HAZLETON EUROPE

FINAL REPORT

Imidan 70-WP: Acute Inhalation Toxicity Study in Rats

Report for:	Gowan Company Box 5569 Yuma Arizona 85366-5569 USA
Data requirement:	USEPA Guideline 81-3
Author:	A Mould
Test facility:	Hazleton Europe Otley Road Harrogate North Yorkshire HG3 1PY ENGLAND
Report number:	1316/1-1050
Sponsor's monitor:	Bethany G Hulcy, Registration Specialist
Report issue:	February 1995

CONFIDENTIALITY STATEMENT

(To be supplied by the sponsor)

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

Company: Gowan Company

Company Agent: Bethany G. Huley Date: 21 Feb 1995

Title: Registration Specialist

Signature: Bethany G Huley

STUDY DIRECTOR AUTHENTICATION AND GLP COMPLIANCE STATEMENT

Imidan 70-WP: Acute Inhalation Toxicity Study in Rats

I, the undersigned, hereby declare that the work described in this report was performed under my supervision. I consider the results to be valid and that the report provides a true and accurate record of the results obtained. The study was performed in accordance with the agreed protocol, and with Hazleton Standard Operating Procedures, unless otherwise stated, and the study objectives were achieved.

This study does not meet the requirements of 40 CFR Part 160 Federal Register, 17 August 1989. This study differs from the requirements of 40 CFR, Part 160 in the following manner: this study was conducted in compliance with the UK Principles of Good Laboratory Practice, the UK Compliance Programme, Department of Health, London 1989.

A P Mould

A P Mould BSc
Study Director

8 Feb 95
Date:

Bethany Goldsby

Applicant/Submitter:

21 Feb 95
Date:

Bethany Goldsby

Sponsor:

21 Feb 95
Date:

RESPONSIBLE SCIENTISTS STATEMENT

Imidan 70-WP: Acute Inhalation Toxicity Study in Rats

I, the undersigned, hereby declare that the analytical procedure described in Appendix 7 was performed under my supervision in accordance with the agreed protocol.

I C Fraser BSc
Formulations Analysis **Date:** 3 Feb 1995

I, the undersigned, hereby declare that I have reviewed this report in conjunction with the Study Director and that the interpretation and presentation of the data in the report are consistent with the results obtained.

C J Collins 3 Feb 95

C J Collins BTech MIBiol
Scientific Reviewer **Date:**

QUALITY ASSURANCE RECORD AND AUTHENTICATION STATEMENT

Imidan 70-WP: Acute Inhalation Toxicity Study in Rats

The study described in this report was subject to audit by the independent HE Quality Assurance Unit as indicated below. The findings of each audit were reported to the Study Director and HE management as prescribed by Standard Operating Procedures.

The report audit was designed to confirm that as far as can be reasonably established the methods described and results incorporated in the final report accurately reflect the raw data produced during the study.

Inspection programme	Inspection date	Report date
Protocol review	17 August 1994	22 August 1994
Data review	October 1994	10 October 1994
Study report (draft)	October 1994	10 October 1994
Study report (final)	February 1995	8 February 1995

Carl Wood

G Wood
Section Head Quality Assurance

Date: 8 February 1995

ARCHIVE STATEMENT

All primary data, or authenticated copies thereof, specimens and the final report will be retained in the Hazleton Europe archives for three years after submission of the final report. At this time the sponsor will be contacted to determine whether data should be returned, retained or destroyed on their behalf.

Specimens requiring storage deep frozen are specifically excluded from the above. These will be retained for as long as the quality of the material permits evaluation but for no longer than three months after submission of the final report. The study sponsor will be notified before specimens are destroyed on their behalf.

Raw data and records pertaining to the toxicokinetic analyses, performed by the sponsor, will be retained and archived by the sponsor.

STUDY IDENTIFICATION

HE Study No:	1316/1
Test article:	Imidan 70-WP
Sponsor/Study Monitor:	Gowan Company Box 5569 Yuma Arizona 85366-5569 USA
Study Director:	A P Mould Hazleton Europe Otley Road Harrogate North Yorkshire ENGLAND HG3 1PY

Study timetable:

Test article receipt:	30 June 1994
Animals arrived:	2 August 1994
Start dosing:	10 August 1994
Completion date:	8 February 1995

RESPONSIBLE PERSONNEL

In addition, the following staff were responsible for key elements of the study

Deputy Study Director	: A C Gibbs
Animal House Supervisor	: T Jameson
Animal House Technician	: J Cunningham
Animal Health	: A Basford
Head of Pathology	: J R Glaister
Inhalation Chemist	: B Canham
Necropsy	: I Wilkins
Histology	: S Brogden

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1 SUMMARY

The objective of the study was to estimate in the rat using head-only exposure the 4 hour acute inhalation median lethal concentration of the test article, Imidan 70-WP, active ingredient phosmet [N-(mercaptomethyl) phthalimide, S-(O,O- dimethyl phosphorodithioate].

Groups of ten rats (five males and five females) of the Crl:CD(SD)BR strain were exposed to the following chamber concentrations by inhalation head-only over a period of four hours:

Group number	Group description	Chamber concentration: Gravimetric method (mg/L 70-WP)	Chamber concentration: GC method mg/L phosmet	Chamber concentration: GC method mg/L 70-WP equivalent
1	control	0	0	0
2	low	1.06	0.46	0.66
3	intermediate	2.08	0.43	0.61
4	high	4.77	2.59	3.69

Exposure was followed by an observation period of 14 days.

The exposure chamber temperature recorded for the control and treated groups ranged between 18 and 22°C, and the chamber relative humidity was between 26 and 65%.

The chamber air flow rates were always 30 L/min for the control and test groups.

The median values for the mean mass median aerodynamic diameter of the particles in the atmosphere ranged from 1.61 to 2.38 μm.

All animals in the high dose were removed from exposure and killed *in extremis*.

Clinical signs were observed on the day of exposure in surviving treated animals. These included lethargy, tremors, coldness to touch, bulging eyes and hunched posture.

After Day 2, low and intermediate dose males gained weight at a lower rate compared with controls, such that by the end of the study, the overall body weight gain was significantly lower than controls. Females gained weight at a similar rate compared with controls, although by the end of the study, the overall body weight gain was still lower than controls.

The absolute and relative lung weights of top dose animals were increased when compared with controls.

All high dose animals had dark or red lungs when they were necropsied on Day 1, which may be related to the mode of death, and does not necessarily indicate that the lung is the target organ.

In conclusion, the acute median lethal concentration (LC_{50}) of the test article, Imidan 70-WP, measured gravimetrically, was calculated to be 3.16 mg/L for males, females and sexes combined. The acute median lethal concentration (LC_{50}) of Imidan 70-WP, determined by GC analysis was calculated to be 1.12 mg/L, expressed in terms of the active ingredient, and 1.6 mg/L, expressed in terms of the formulated product, for males, females and sexes combined.

2 INTRODUCTION

The objective of the study was to estimate the 4 hour acute inhalation median lethal concentration of the test article, Imidan 70-WP, in the rat using head-only exposure.

The inhalation route was chosen as it is a possible route of human exposure.

The rat was selected as it is a readily available rodent species acceptable to the regulatory authorities with documented susceptibility to a wide range of toxic substances. Background data are available on the selected strain.

The study was conducted in accordance with the requirements of US EPA guideline 81-3 for the testing of pesticides.

The study was preceded by a preliminary exposure in which one male and one female rat were exposed to a mean gravimetric atmosphere concentration of 5.06 mg/L (analysed by gas chromatography as 2.50 mg/L active ingredient, phosmet). Both animals died during the exposure period, the male after 2 hours 48 minutes, and the female after 3 hours 15 minutes from the start of the exposure period. As a result of this exposure, it was decided to proceed with the initial exposure of the main study at the target limit concentration of 5 mg/L of 70-WP formulation.

The results of the preliminary study are retained in the data file but not reported further.

The animals were received by HE on 2 August 1994. Exposure started on 10 August 1994 and the necropsies were completed on 6 September 1994.

3 MATERIALS AND METHODS

3.1 Protocol adherence

The study was conducted in accordance with the agreed protocol, number P9368d, presented in Appendix 8. There were no major deviations from the protocol. Minor deviations, which did not affect the integrity or outcome of the study, are also presented in Appendix 8.

3.2 Test and control articles

3.2.1 Description, identification and storage

The test article, Imidan 70-WP, a beige powder, was received at HE as follows:

Batch number	Quantity (g)	Purity (%)	Date of receipt at HE
06213080	1500	70.2	30 June 1994

After receipt, but prior to the start of the study, the test article was sent for micronisation to Micron Mills Limited, Orpington, Kent, UK, where it was micronised by fluid energy milling. The material was passed through the mill several times until the material was as small as possible. After each pass through the mill, the micronised material was measured using a Malvern 2600 Particle Sizer, using a method developed at Micron Mills. The test material was passed through the mill 4 times. The estimated median diameter of the successive runs were 7.87, 8.05, 7.19 and 6.35 μm. The Malvern printouts of the individual analyses of the micronisations are retained on file at HE, but are not reported further here.

The test article contained 70.2% of active ingredient. The results of an analysis of the micronised test article used in this study are presented in Appendix 5.

The stability of the test article under the conditions used was confirmed by the sponsor, and the expiry date was stated to be October 1996.

When not in use the test article was stored at ambient temperature, over desiccant.

After micronisation, the test article was used as supplied.

The control article was room air filtered prior to ingress to the room.

3.2.2 Route and method of administration

The test article was administered by head-only inhalation for a period of four hours.

3.2.3 Experimental design and dose levels

The following groups were exposed:

Group number	Chamber concentration: Target concentration (mg/L 70-WP)	Nominal concentration (mg/L 70-WP)	Gravimetric (mg/L 70-WP)	GC Method: mg/L phosmet	GC Method: mg/L 70-WP equivalent
2	1	1.72	1.06	0.46	0.66
3	2	2.77	2.08	0.43	0.61
4	5	6.21	4.77	2.59	3.69

Group 1, exposed under similar conditions to an atmosphere of filtered air, acted as a control.

The day of exposure was followed by a 14 day observation period. The exposure of test and control groups was conducted on different days and the necropsy days were staggered accordingly.

3.3 Test system

3.3.1 Species, strain and supplier

A sufficient number of rats of the Crl:CD(SD)BR strain was obtained from Charles River (UK) Ltd, Margate, to provide 20 healthy animals of each sex.

3.3.2 Specification

The animals were ordered to be in the age range 6 to 7 weeks old on arrival. All animals were given an external examination for signs of ill health on

arrival. The animals were acclimatised to the holding room for at least five days during which time their health status was reassessed and their suitability for experimental purposes confirmed.

3.3.3 Environment and husbandry

The animals were housed in a single room air-conditioned to provide a minimum of 15 air changes/hour and routinely maintained at a temperature of 19 to 25°C and a relative humidity of 40 to 70%. Fluorescent lighting was controlled automatically to give a cycle of 12 hours light (0600 to 1800 h) and 12 hours darkness.

The animals were caged in groups of five in stainless steel wire mesh cages suspended over cardboard-lined trays. The cardboard liners were replaced as often as was necessary to maintain hygienic conditions.

3.3.4 Diet and water

Except during the exposure period, animals had free access to SQC Rat and Mouse Maintenance Diet No 1, Expanded (Special Diets Services Ltd, Witham).

Mains drinking water was available *ad libitum*, except during the exposure period, from water bottles attached to the cages. The contents of the bottles were changed daily.

The diet and water were considered not to have contained any contaminant at a level which might have affected the integrity or outcome of the study.

3.4 Allocation to treatment group

Animals were assigned, sexes separately, as they came to hand on arrival, one to each cage for the available cages, until each cage contained the required number. Cages were arbitrarily allocated to study.

3.5 Identification of the test system

After allocation to treatment group each animal was permanently numbered by indelible ink on the tail as follows:

Group number	Colour code	Animal identification numbers	
		Male	Female
1	buff	1 - 5	6 - 10
2	blue	21 - 25	26 - 30
3	yellow	31 - 35	36 - 40
4	pink	11 - 15	16 - 20

A colour-coded card on each cage gave information including study number and animal number.

3.6 Production of test atmosphere

A schematic diagram of the continuous flow system used is shown in Appendix 3 of the protocol (Annex). The compressed air supply used was breathing quality air.

An RBG 1000 powder generator located immediately above the chamber, and supplied with compressed air, was used to produce the atmospheres of test article.

The atmosphere was introduced into the top of the cylindrical aluminium exposure chamber (approximately 40 L internal volume).

Chamber air flow rates were monitored continuously and recorded at half-hourly intervals. The test atmospheres were filtered using a cartridge particulate filter, exhausted to the outside of the building and vented.

3.7 Atmosphere control

3.7.1 Exposure chamber temperature and relative humidity

The temperature and relative humidity inside the exposure chamber were measured continuously and recorded at half-hourly intervals throughout the four hour exposure period, using a digital thermometer with a remote probe

located inside the chamber, and a paper hygrometer located in the exhaust duct of the chamber.

3.7.2 Exposure chamber oxygen concentration

The oxygen concentration inside the chamber was monitored continuously and recorded at half-hourly intervals throughout the four hour exposure period using an oxygen analyser incorporating a remote sensor.

3.7.3 Chamber air flow

Chamber air flow was monitored continuously and recorded at half-hourly intervals throughout the four hour exposure period.

There were approximately 45 air changes/hour.

3.7.4 Measured concentration

The concentration of the test article was determined gravimetrically. The samples were obtained twice hourly, over periods of 2 minutes, during exposure.

The atmosphere was sampled by drawing a known volume through a glass fibre open face filter positioned at a site representative of that occupied by the external nares of the experimental animals. The filter was weighed before and after sampling and the measured concentration of the test atmosphere calculated as follows:

$$\text{Gravimetric concentration (mg/L)} = \frac{\text{total weight gain (mg)}}{\text{volume of sample (L)}}$$

The particulate trapped on the filters at each sampling period was also analysed by gas chromatography (GC) for phosmet. The analytical method is presented as Appendix 7.

The analysed results were subsequently used to calculate the atmosphere concentrations expressed in terms of formulated product as follows:

$$\text{Concentration in terms of formulated product (mg/L 70-WP)} = \text{Analysed concentration (mg/L)} \times \frac{100}{70.2}$$

3.7.5 Nominal concentration

Nominal concentration is defined as the amount of test article released per minute from the generator divided by the airflow/minute passing through the exposure system. Units of measurement are routinely mg/L. The total weight of test article used and the volume of diluent air used to generate each test atmosphere were recorded and the nominal concentration of the test article in the exposure chamber was calculated as shown:

$$\text{Nominal concentration (mg/L)} = \frac{\text{weight of test article used (mg)}}{\text{air flow (L/min) x duration (min)}}$$

3.7.6 Exposure chamber particle size analysis

The particle size was determined using an Andersen 298 Marple Cascade Impactor, with six separation stages corresponding to maximum mass median aerodynamic diameters of 0.52, 0.93, 1.55, 3.50, 6.00 and 9.80 μm. The samples were obtained hourly over periods of up to 2 minutes, during each exposure period (ie during hours 1, 2, 3 and 4).

The cumulative percentage by weight of test article collected at each successive stage was plotted by computer as a probability value against the logarithmic value of the upper class limit of that stage. The point at which the cumulative distribution line crossed the 50 percentile was the estimate of the mass median aerodynamic diameter (MMAD). The Geometric Standard Deviation (GSD) was also calculated.

The percentage by mass of particles less than 1 μm mass median aerodynamic diameter was estimated based on the weight of test article collected on each stage of the impactor.

3.8 Experimental observations

3.8.1 Clinical signs

The animals were observed at hourly intervals during the exposure period, for the remainder of the working day and once daily thereafter for 14 days. An individual record was maintained of the clinical condition of each animal.

3.8.2 Morbidity and mortality

All animals were examined twice daily to detect any which were dead or moribund. Moribund animals were removed and necropsied to prevent autolysis.

3.8.3 Body weight

The body weight of each animal was recorded immediately before and after exposure and on Days 2, 8 and 15 of the study, and at necropsy.

3.9 Pathology

The following procedures were applied to all animals killed at the end of the study, and to those killed *in extremis*:

3.9.1 Necropsy

The animals were given an intraperitoneal injection of sodium pentobarbitone. Following exsanguination a full internal and external examination was made under the general supervision of a pathologist and all lesions were recorded. This examination included an assessment of any irritation of the respiratory tract.

3.9.2 Lung weights

The lungs were weighed before fixation.

3.9.3 Histology

All gross lesions were fixed in 10% neutral buffered formalin and retained without further processing.

3.10 Data presentation

Data were processed, where appropriate, to give group mean values and standard deviations.

3.11 Statistical evaluation

Median lethal concentration (LC_{50}) values were calculated from the doses measured gravimetrically, and measured using gas chromatography, expressed in terms of the active ingredient, and in terms of the formulated product.

The LC_{50} was calculated separately for males and females from the recorded mortality rate using a probit analysis method (Finney, DJ (1971), Probit Analysis, 3rd ed Cambridge University Press). The LC_{50} was also calculated for the sexes combined. The probit analysis method used has not undergone separate validation by Hazleton Europe.

The statistical data are presented as Appendix 6.

4 RESULTS

4.1 Exposure chamber temperature and relative humidity (Table 1 and Appendix 1)

The temperature recorded for the control and treated groups ranged between 18 and 22°C and the relative humidity between 50 and 52% for the control group, and between 26 and 65% for the treated groups.

4.2 Exposure chamber diluent air flow rates (Table 1 and Appendix 1)

The flow rates were always 30 L/min for the control and test groups.

4.3 Exposure chamber oxygen concentration (Table 1 and Appendix 1)

The oxygen concentration was 20.4 to 21.1% for the control and treated groups.

These values were considered to be satisfactory.

4.4 Measured exposure chamber atmosphere concentrations (Table 2)

The mean concentrations of phosmet in the generated atmospheres, measured by gas chromatography were 0.46, 0.43 and 2.59 mg/L. Expressed in terms of the formulated product, Imidan 70-WP, these were calculated to be 0.66, 0.61 and 3.69 mg/L respectively. The corresponding gravimetric and nominal concentrations were 1.06, 2.08 and 4.77 mg/L, and 1.72, 2.77 and 6.21 mg/L. The proportionality between the gravimetric and nominal concentrations (the efficiency of generation) was in the range 61 to 77%.

4.5 Exposure chamber particle size analysis (Table 3, Figures 3 to 8)

The median values for the mean mass median aerodynamic diameter of the particles in the atmosphere for the various groups were 1.61 to 2.38 μm. The corresponding values for the geometric standard deviation were 1.95 to 2.04. This resulted in approximately 11 to 27% of the atmosphere having an aerodynamic equivalent diameter (AED) of 1 μm or less. These values indicate that the mean diameter of the aerosol droplets was well within the respirable range of the rat (up to 5 μm).

4.6 Mortality (Table 4)

All high dose animals were removed from exposure between approximately 2½ and 3½ hours and killed *in extremis*, because they ran the risk of suffering excessive distress.

There were no deaths in any of the other groups.

4.7 Clinical observations

Before being removed from exposure and terminated, the only significant clinical sign in high dose animals was laboured respiration, which was seen in all animals.

During exposure, clinical signs observed in low and intermediate dose animals were chromodacryorrhea and wet fur. However, on Day 1 after exposure, clinical signs observed in these two groups included lethargy, tremors, coldness to touch, bulging eyes, hunched posture, piloerection and stained fur around the nose and mouth.

The majority of the clinical signs had resolved by Day 2, apart from lethargy, which was not seen after Day 2; hunched posture, which persisted, and was still observed on Day 7 in low dose animals and on Day 10 in intermediate dose animals; and stained fur, which persisted and was still seen on Days 14 and 15 in low and intermediate dose animals respectively.

Other minor clinical signs seen on the day of exposure in the control, low and intermediate dose groups, were attributable to the method of restraint. These included wet fur and chromodacryorrhea. Chromodacryorrhea was seen until Days 3 and 8, in low and intermediate dose animals respectively.

4.8 Body weight (Figures 1 and 2, Table 5, Appendix 2)

Small body weight losses occurred as a result of the restraint procedure in the control and surviving treated groups.

Although control and low dose animals began to gain weight on Day 2, compared to their post-exposure body weight, intermediate dose animals lost weight on Day 2. Thereafter, intermediate dose females gained weight at a similar rate compared with

controls, such that by the end of the study the overall body weight gain of this group was lower than controls.

After Day 2, low and intermediate dose males gained weight at a lower rate compared with controls, such that by the end of the study the overall body weight gain was significantly lower than controls.

4.9 Lung weights (Table 6, Appendix 3)

There was a small increase in absolute lung weights of high dose animals when compared with controls. The relative lung weights of the same animals were significantly increased when compared with controls, probably associated with congestion of the airways, as a result of the laboured respiration observed in these animals. There were no significant differences between the lung weights of low and intermediate dose animals when compared with controls.

4.10 Macroscopic pathology (Appendix 4)

The only necropsy finding which was considered to be significant was dark or red lobes, which was seen in all high dose animals at necropsy on Day 1. This finding was consistent with the absolute and relative lung weight effects of this group. The necropsy findings may be related to the mode of death, and do not necessarily indicate that the lung is the target organ.

There were no other findings that were suggestive of test article toxicity.

4.11 Acute median lethal concentration (Appendix 6)

Median lethal concentration (LC_{50}) values were calculated from the doses measured gravimetrically, and measured using gas chromatography, expressed in terms of the active ingredient, and in terms of the formulated product.

The LC_{50} based on gravimetric results was 3.16 mg/L for males and females only and for sexes combined. The LC_{50} based on GC results was 1.12 mg/L, in terms of the active ingredient, and 1.6 mg/L, in terms of the formulated product, for males and females only and for sexes combined.

The 95% fiducial limits were not calculable from the data.

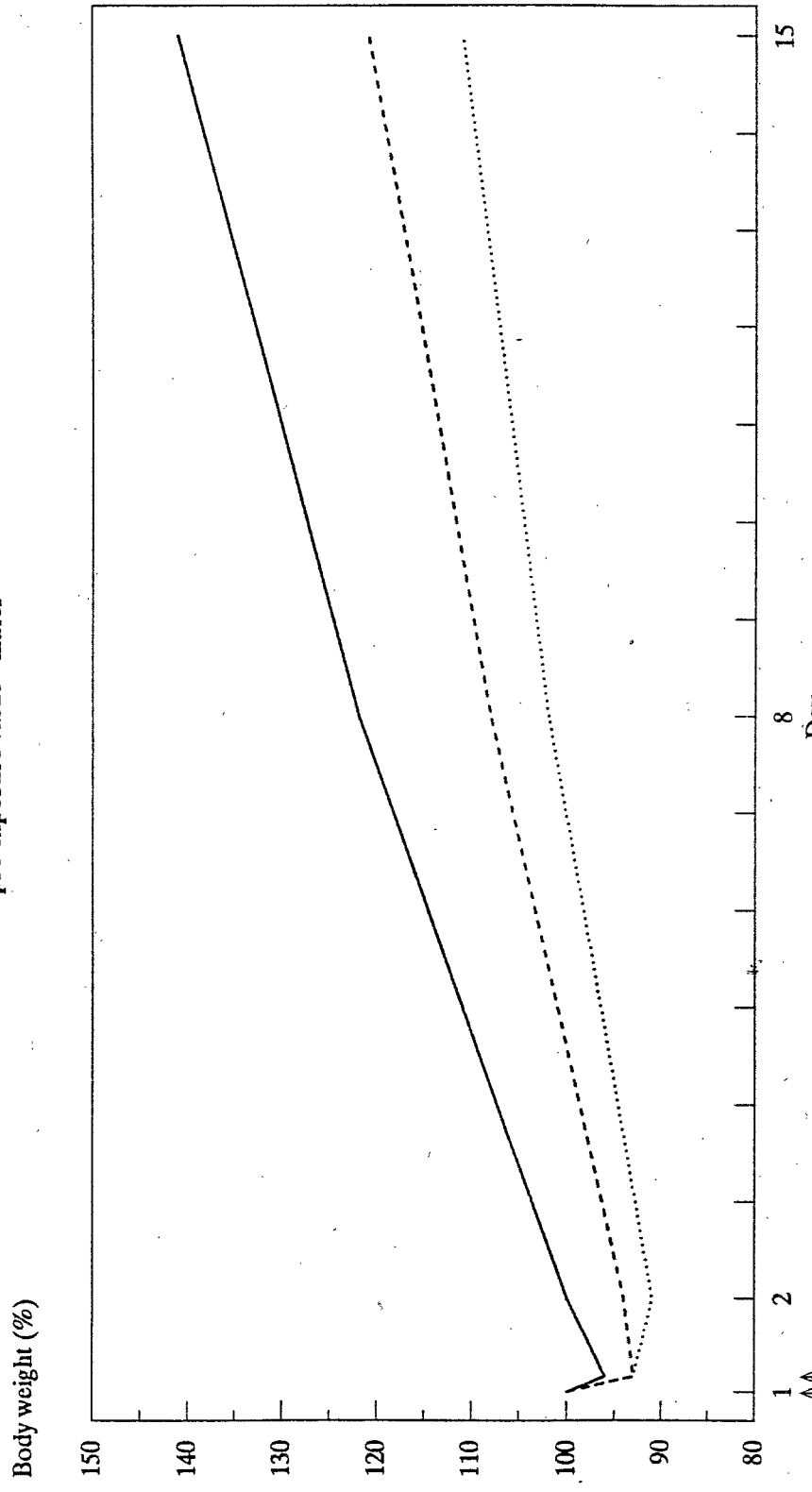
5 DISCUSSION AND CONCLUSION

The mean analysed concentrations of phosmet, expressed in terms of the formulated product, represented approximately 62, 29 and 77% of the gravimetric results for the low, intermediate and high dose groups respectively. A possible explanation for the difference between the two sets of values (those for formulated product and those measured gravimetrically) is adsorption of water vapour by the test article and by the glass fibre filters. This would give an effect in the observed direction (ie to increase the gravimetric values); since there was less test article collected on the low and intermediate group filters compared with the high dose group, the adsorption of water vapour would have a proportionally greater effect.

However, the analytical result for the intermediate dose group was significantly lower than the corresponding gravimetric result, and was actually lower than the analytical result for the low dose group. There was no apparent explanation for this anomaly, and as such it can only be considered to be a real value. Since analytical, rather than gravimetric, results are generally regarded as the more accurate estimates of the atmosphere concentration, and in view of the possible adsorption of water vapour, the analytical results, expressed in terms of the formulated product, are considered to be the most accurate descriptors of the results obtained. For this reason, these results were also used to calculate the acute median lethal concentration.

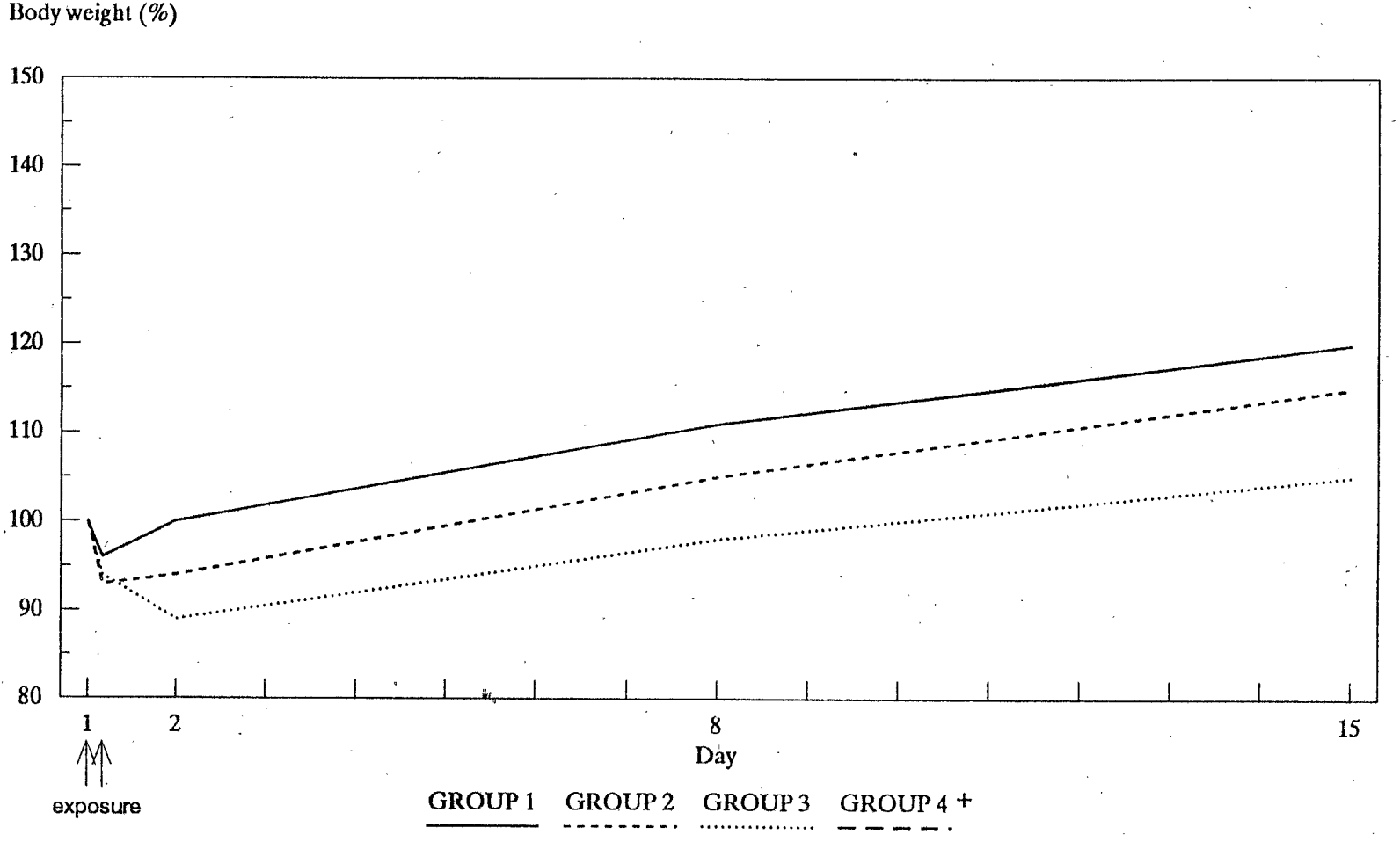
In conclusion, the acute median lethal concentration (LC_{50}) of the test article, Imidan 70-WP, measured gravimetrically, was calculated to be 3.16 mg/L for males, females and sexes combined. The acute median lethal concentration (LC_{50}) of Imidan 70-WP, determined by GC analysis was calculated to be 1.12 mg/L, expressed in terms of the active ingredient, and 1.6 mg/L, expressed in terms of the formulated product, for males, females and sexes combined.

FIGURE 1
Group mean body weight as a percentage of pre-exposure value - males



+ all Group 4 animals died during exposure

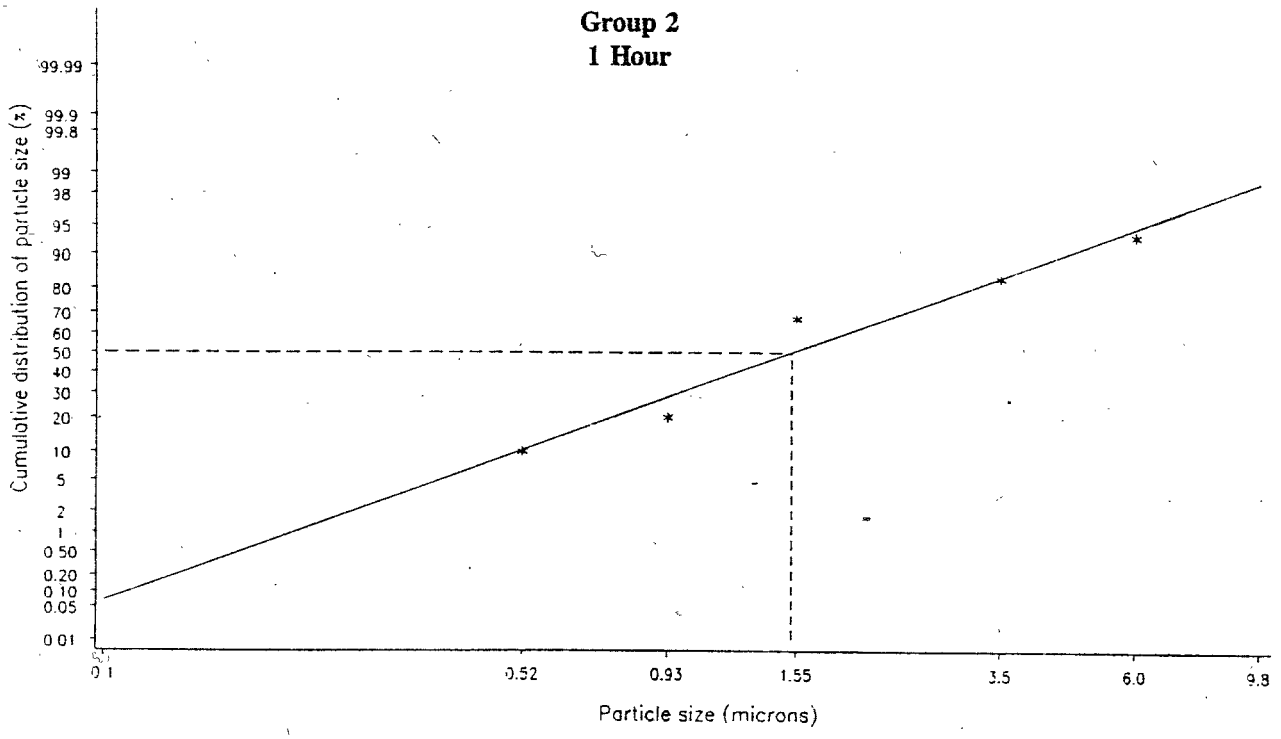
FIGURE 2
Group mean body weight as a percentage of pre-exposure value - females



+ all Group 4 animals died during exposure

FIGURE 3

Plot of Particle Size Distribution
Group 2
1 Hour



Plot of Particle Size Distribution
Group 2
2 Hour

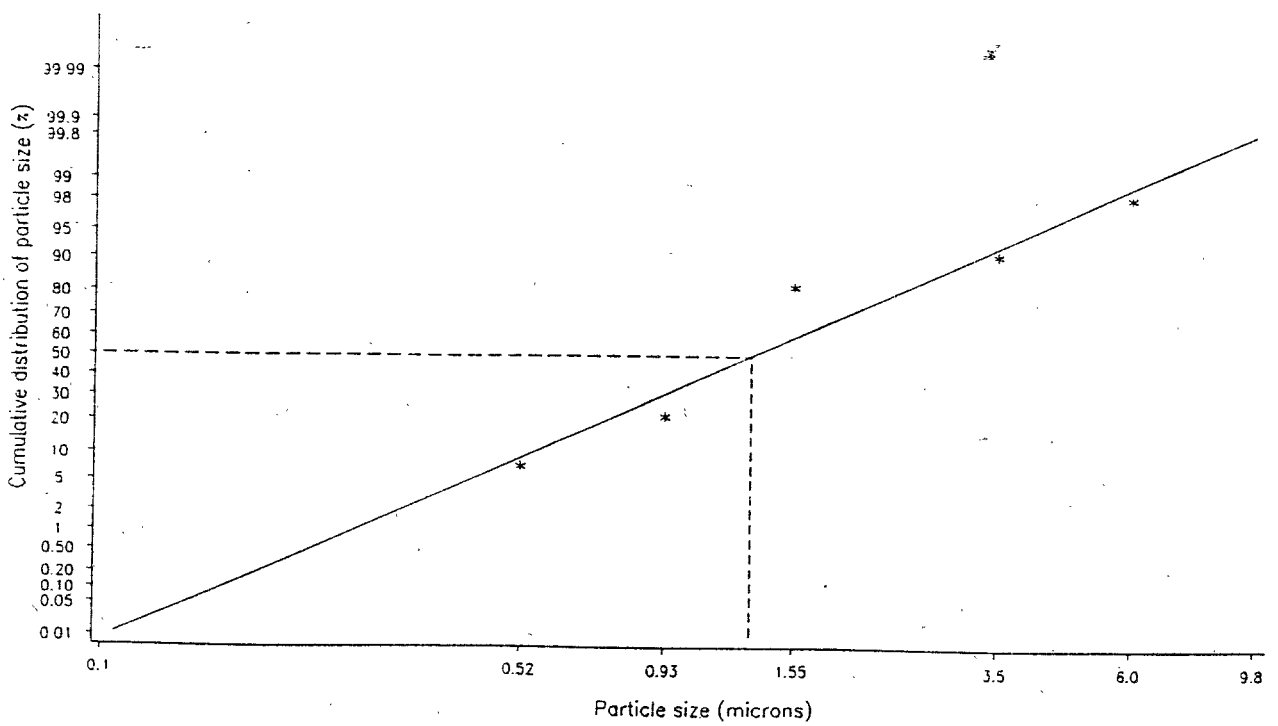
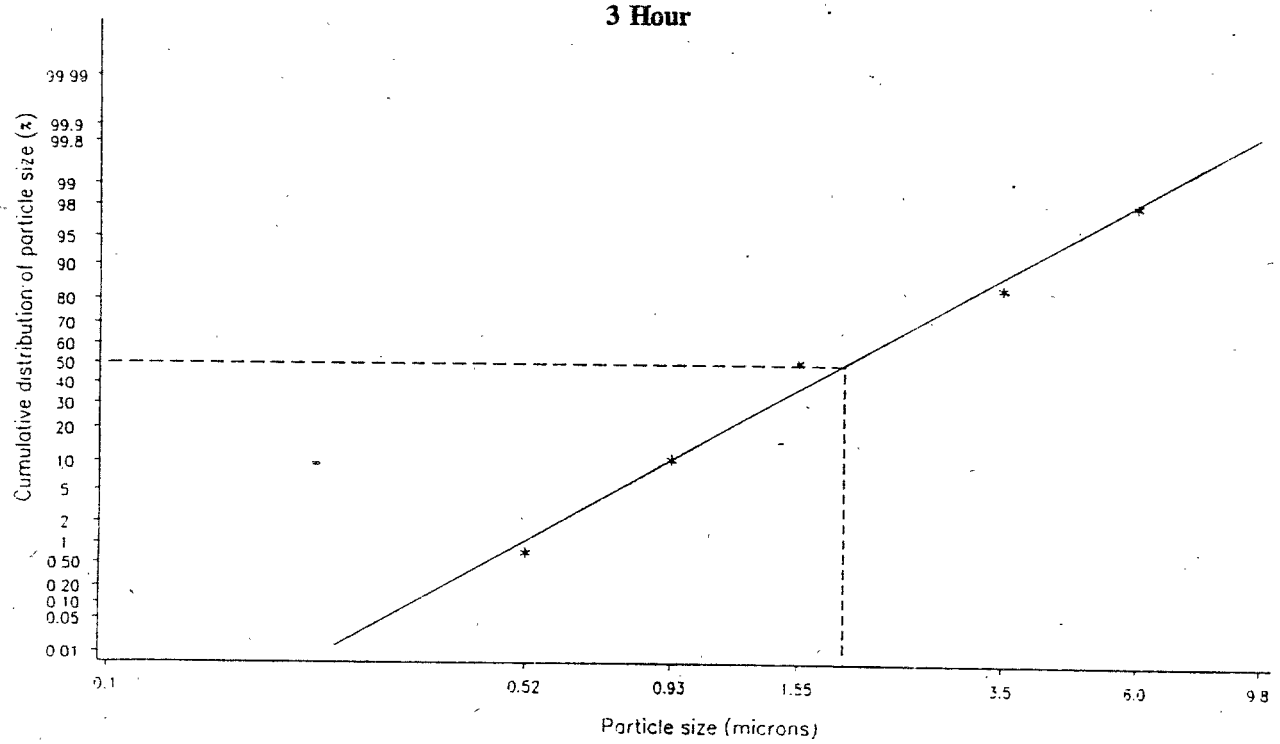


FIGURE 4

Plot of Particle Size Distribution
Group 2
3 Hour



Plot of Particle Size Distribution
Group 2
4 Hour

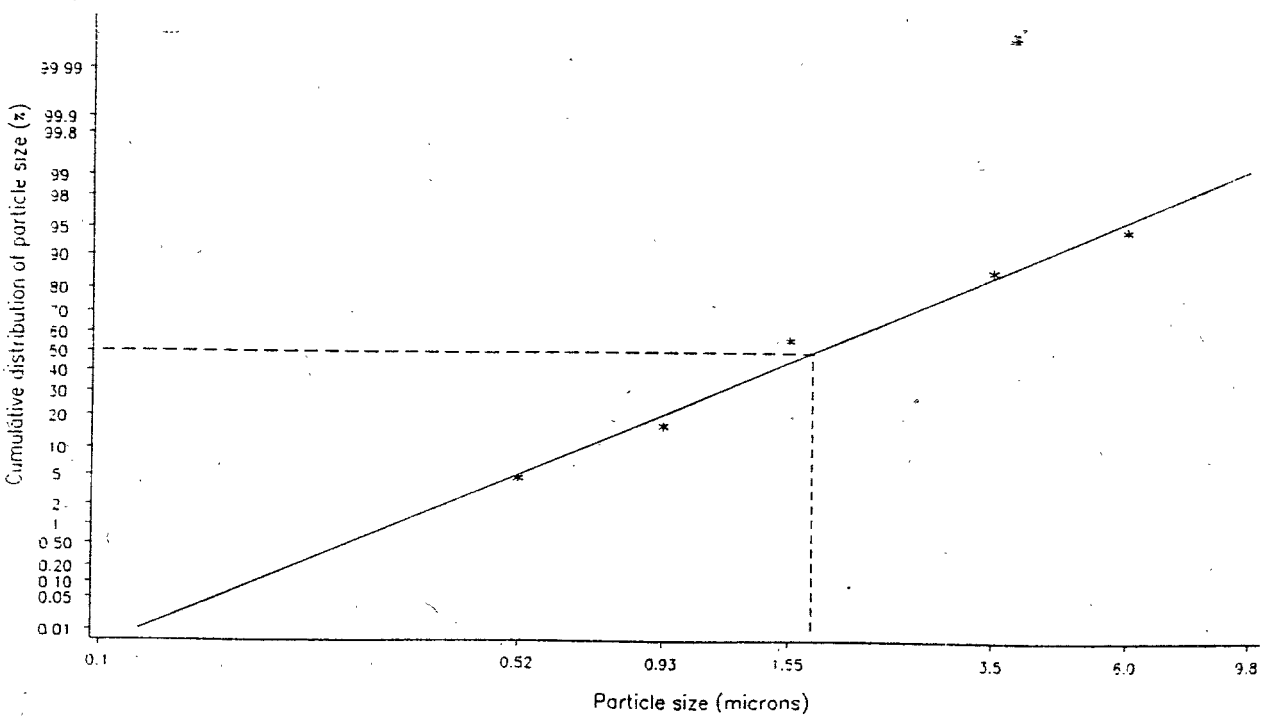
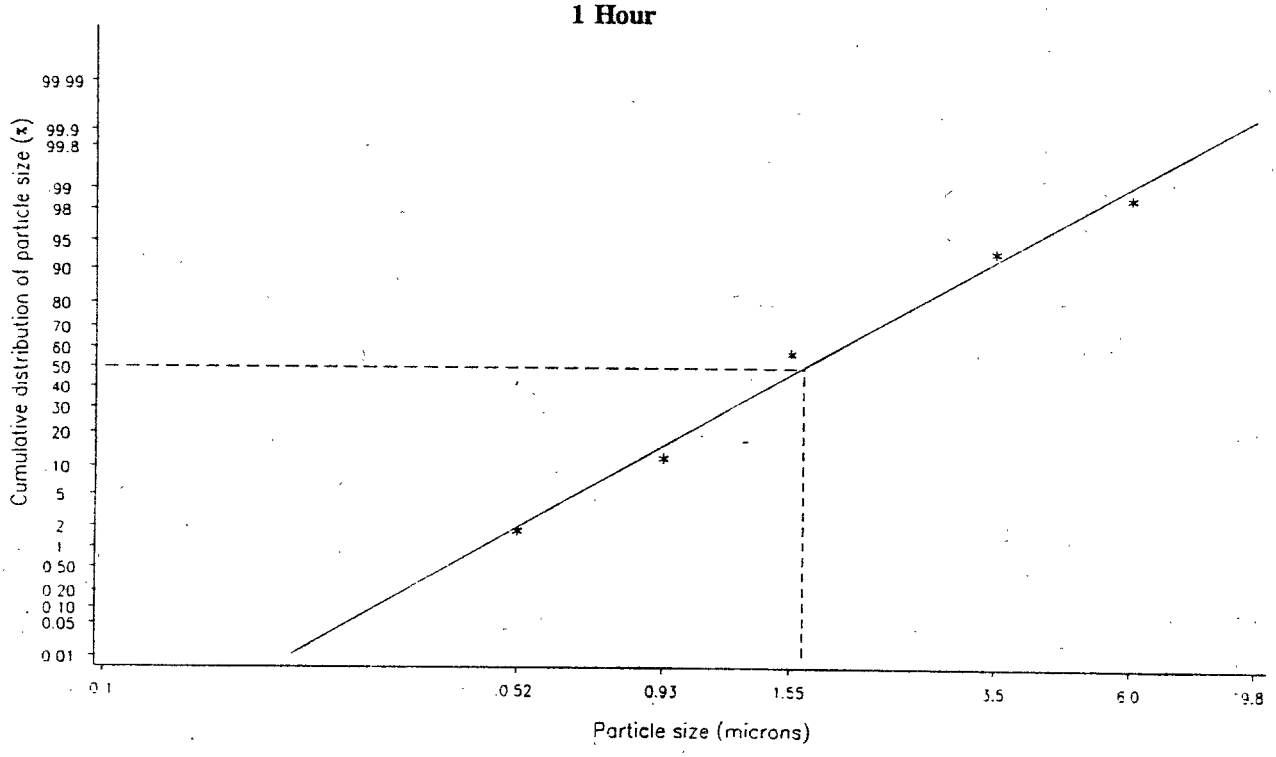


FIGURE 5

Plot of Particle Size Distribution
Group 3
1 Hour



Plot of Particle Size Distribution
Group 3
2 Hour

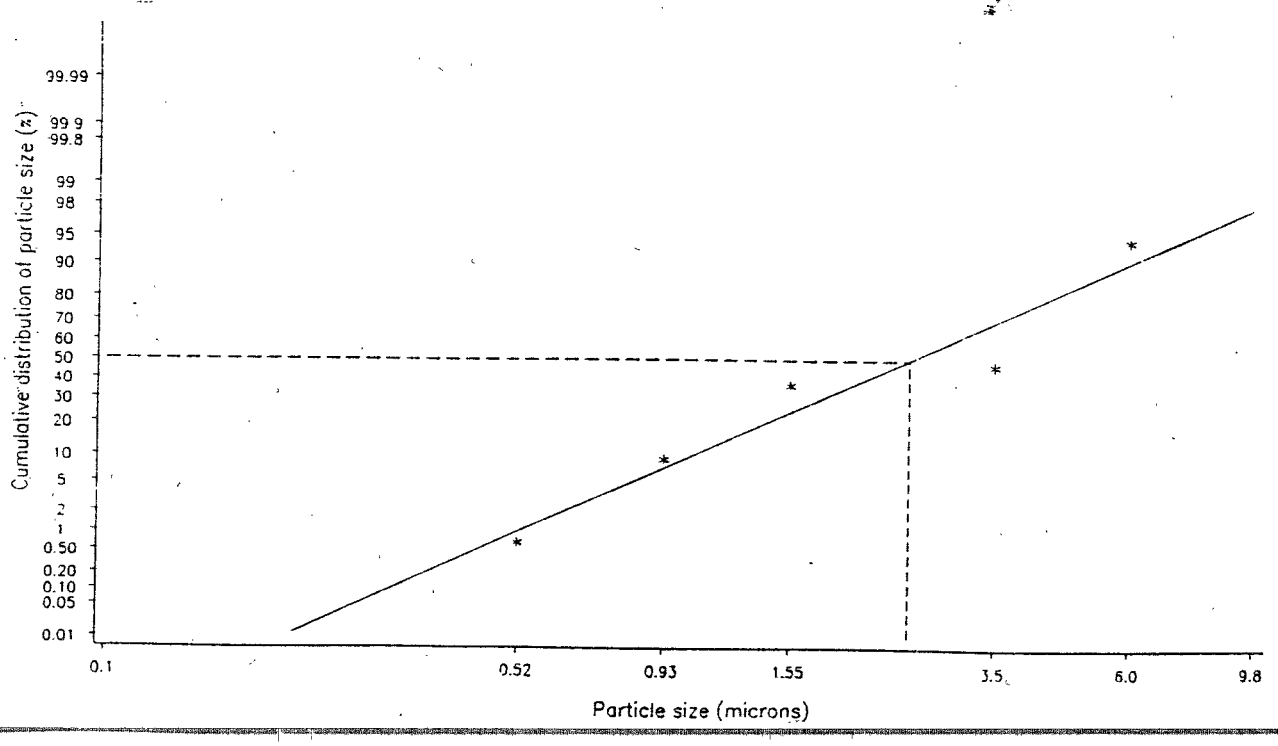
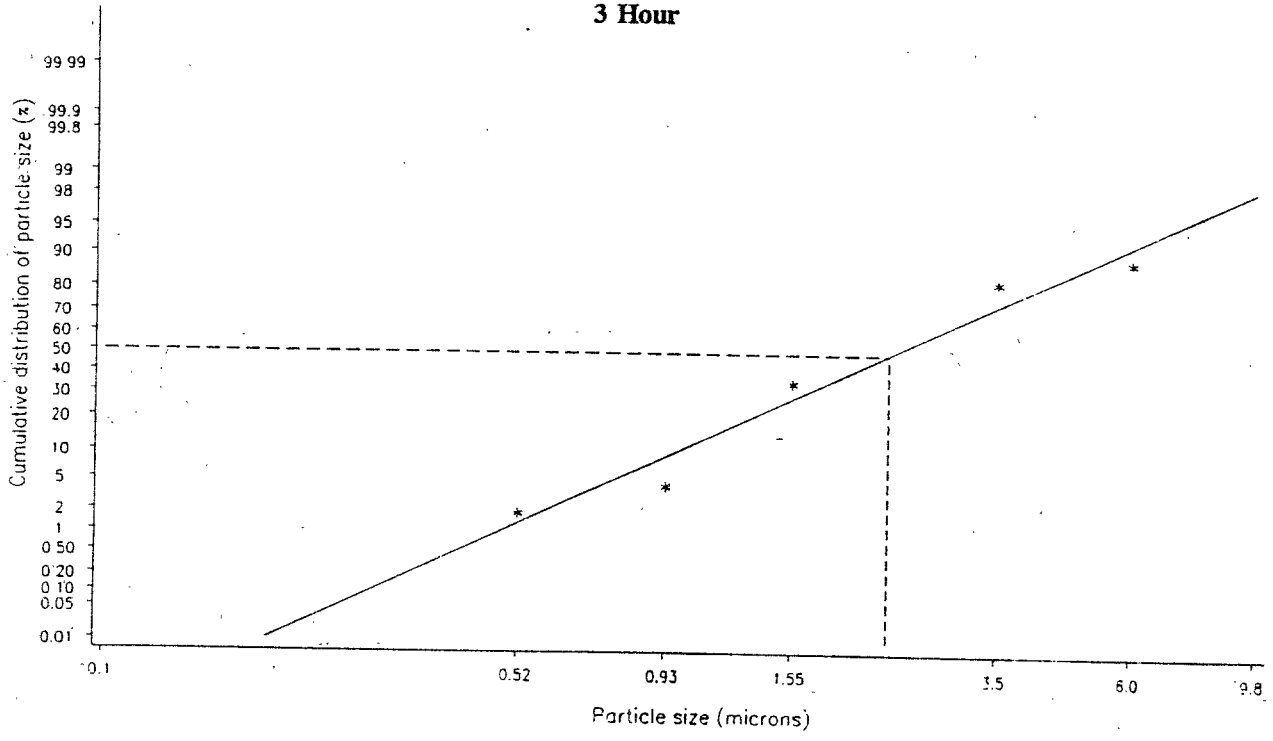


FIGURE 6

Plot of Particle Size Distribution
Group 3
3 Hour



Plot of Particle Size Distribution
Group 3
4 Hour

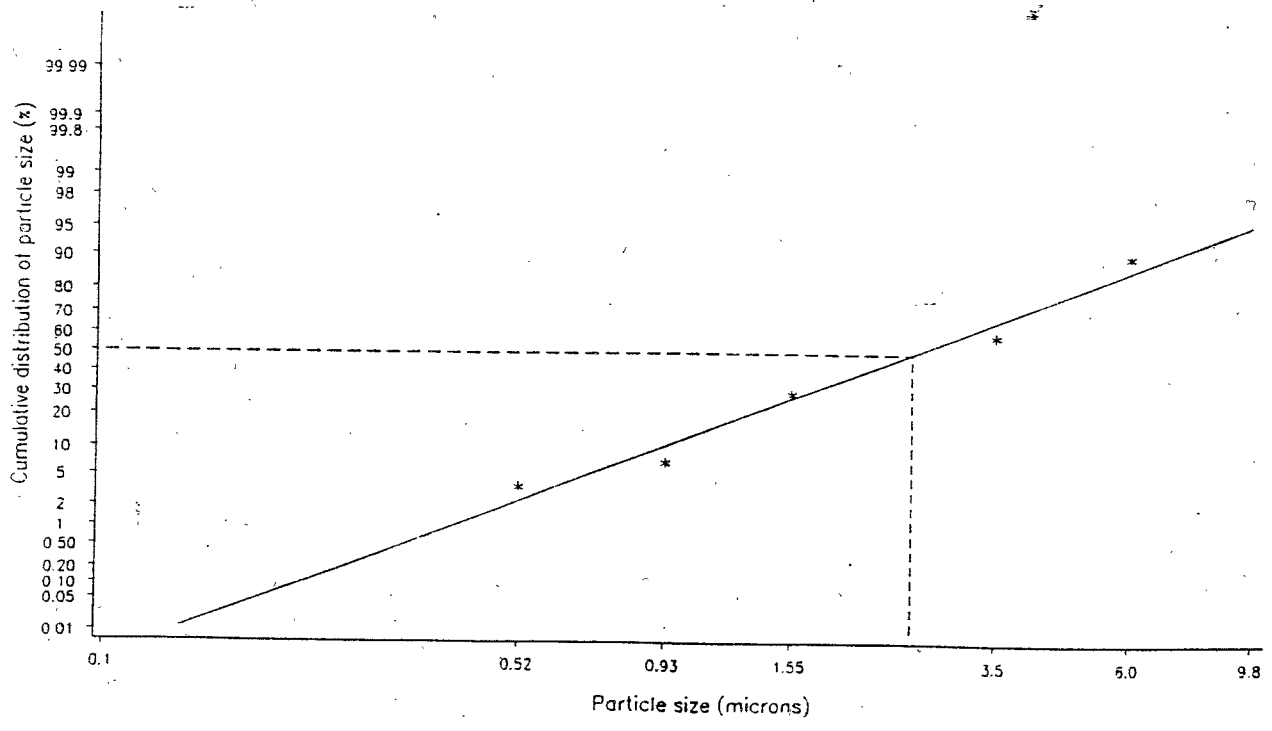
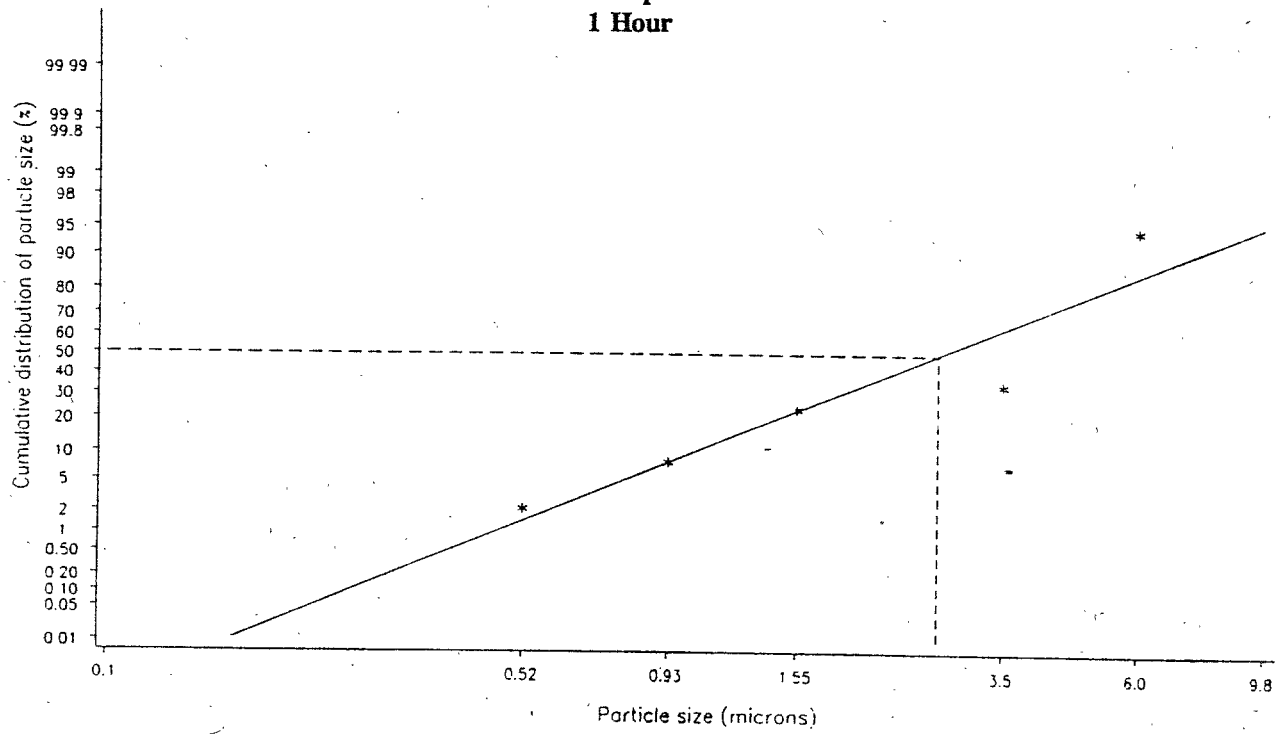


FIGURE 7

Plot of Particle Size Distribution
Group 4
1 Hour



Plot of Particle Size Distribution
Group 4
2 Hour

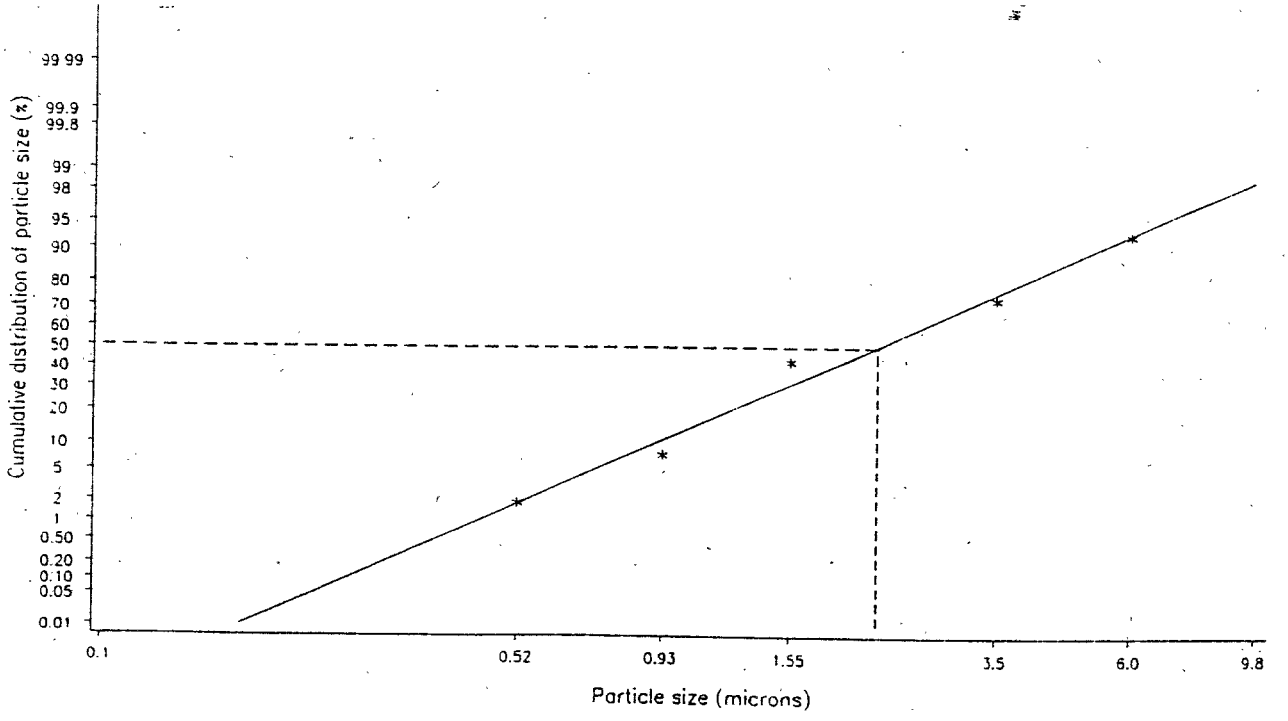
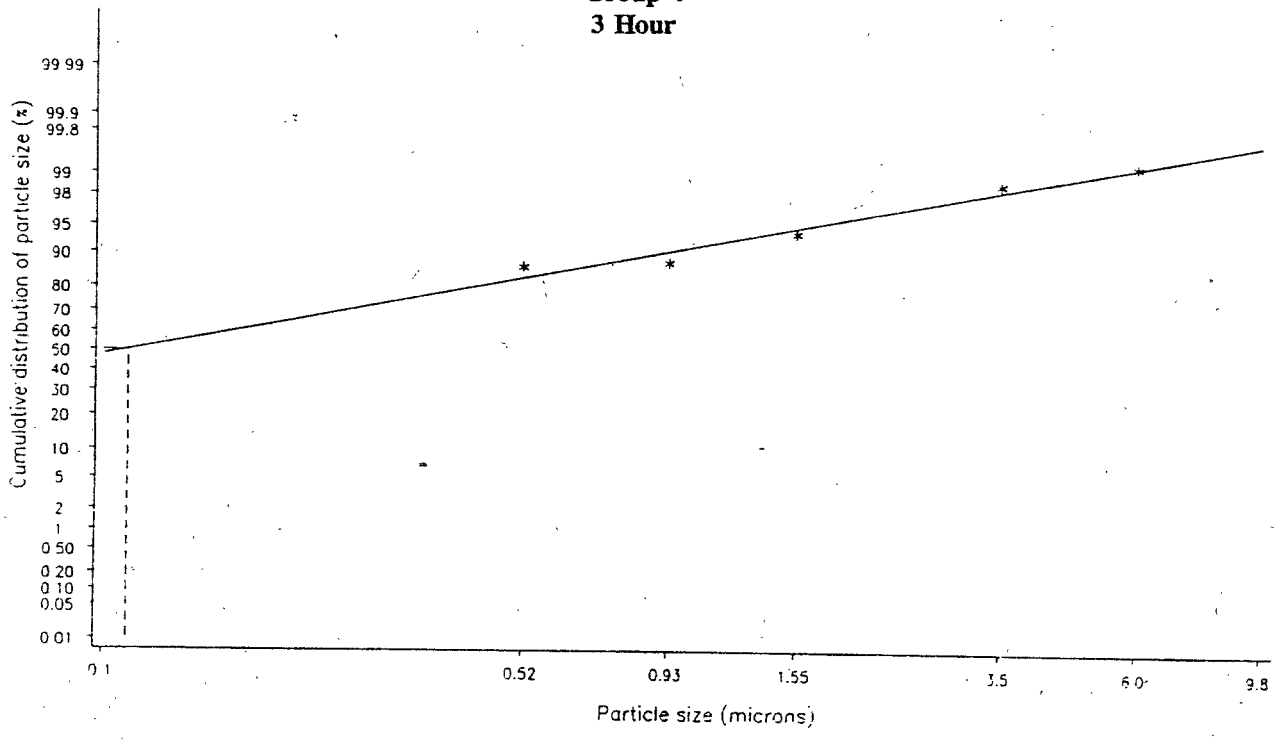


FIGURE 8

Plot of Particle Size Distribution
Group 4
3 Hour



Plot of Particle Size Distribution
Group 4
4 Hour

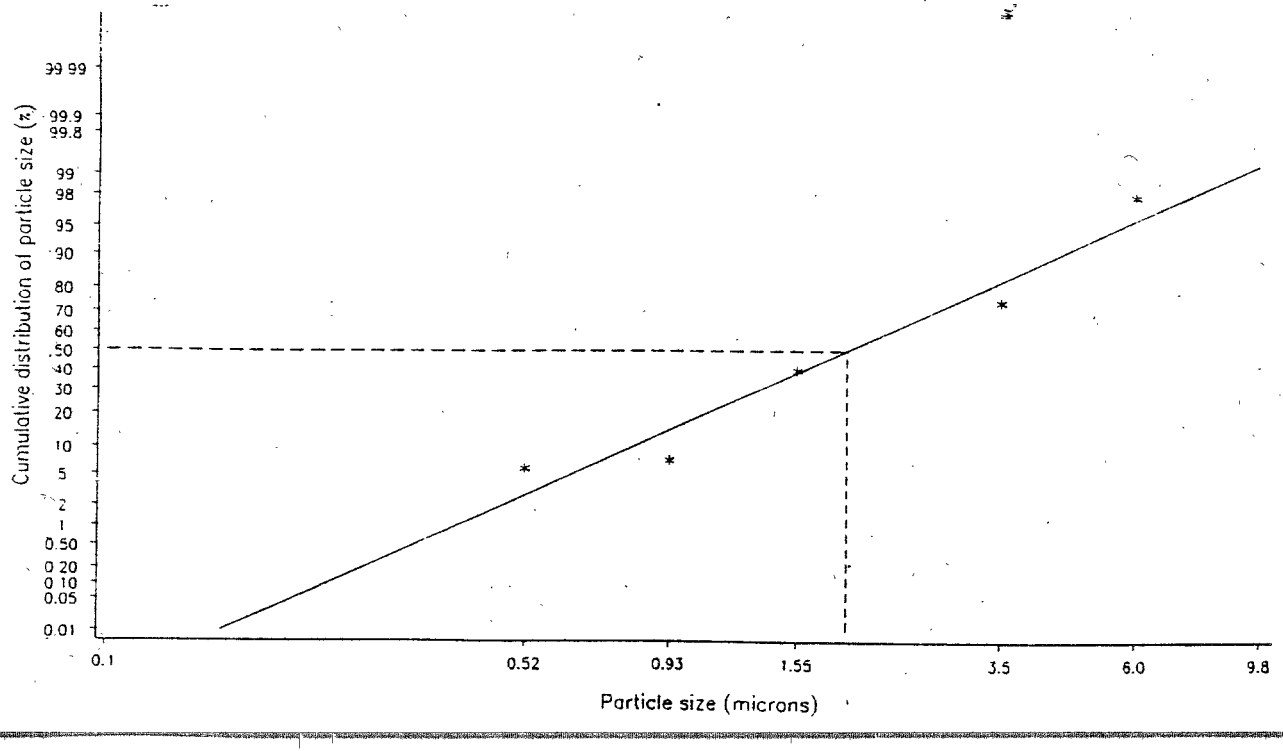


TABLE 1

Mean exposure chamber conditions

Group		Temperature °C	Relative humidity %	Extract air flow L/min	Oxygen concentration %
1	Mean	21	50	30	20.9
	SD	1	1	0	0.1
2	Mean	20	32	30	20.5
	SD	1	4	0	0.1
3	Mean	21	34	30	20.6
	SD	0	12	0	0.1
4	Mean	20	30	30	21.0
	SD	2	6	0	0.1

TABLE 2

Exposure chamber test article concentration (mg/L)

Group	Nominal concentration mg/L		Concentration (mg/L) in hour: ½	1	1½	2	2½	3	3½	4	Mean measured concentration ± SD
2	1.72	Gravimetric	0.17	2.03	1.00	1.23	1.27	0.98	0.90	0.90	1.06 ± 0.52
		Phosmet	ND	0.50	0.29	0.59	0.86	0.31	0.73	0.42	0.46 ± 0.27
3	2.77	Gravimetric	0.44	3.37	2.02	2.72	1.38	1.53	3.02	2.14	2.08 ± 0.96
		Phosmet	0.33	0.34	0.32	0.81	0.68	0.38	0.33	0.27	0.43 ± 0.20
4	6.21	Gravimetric	5.19	5.19	4.47	5.03	5.61	5.43	3.16	4.06	4.77 ± 0.82
		Phosmet	2.11	3.36	2.96	2.62	3.77	2.99	2.27	0.65	2.59 ± 0.95

ND = None detected, included as 0 in calculation of mean

TABLE 3

Exposure chamber particle size analysis

Group		Particle size (μm) in hour: 1	2	3	4	Median value
2	MMAD	1.52	1.31	1.86	1.70	1.61
	GSD	2.31	1.96	1.74	2.04	2.00
	% less than 1 μm diameter	31	34	13	23	27
3	MMAD	1.63	2.49	2.27	2.50	2.38
	GSD	1.73	1.95	1.94	2.19	1.95
	% less than 1 μm diameter	19	9	11	12	12
4	MMAD	2.70	2.17	0.11	1.89	2.03
	GSD	2.11	1.97	5.06	1.93	2.04
	% less than 1 μm diameter	9	13	91	17	15

MMAD = Mass median aerodynamic diameter (μm)
GSD = Geometric standard deviation

TABLE 4

Group mortality

Group and sex	Number animals in group	Mortality on Day 1	Total number deaths
1M	5	0	0
2M	5	0	0
3M	5	0	0
4M	5	5	5
1F	5	0	0
2F	5	0	0
3F	5	0	0
4F	5	5	5

No deaths occured on any other day

TABLE 5

Group mean body weight as a percentage of pre-exposure value

Group and sex		% Pre-exposure on Day: 1 Pre-exposure	1 Post-exposure	2	8	15
1M	Mean	100	96	100	122	141
	SD	0	1	2	4	7
2M	Mean	100	93	94	108	121
	SD	0	1	4	3	3
3M	Mean	100	93	91	102	111
	SD	0	1	3	3	2
4M	Mean	100	Dead			
	SD	0	Dead			

Group and sex		% Pre-exposure on Day: 1 Pre-exposure	1 Post-exposure	2	8	15
1F	Mean	100	96	100	111	120
	SD	0	2	4	3	6
2F	Mean	100	93	94	105	115
	SD	0	1	4	2	2
3F	Mean	100	94	89	98	105
	SD	0	2	5	3	3
4F	Mean	100	Dead			
	SD	0	Dead			

TABLE 6

Group mean lung weights (g) and lung/body weight ratios (%)

Group and sex		Body weight (g)	Lung weight (g)	Ratio (%)
1M	Mean	337.3	1.827	0.5430
	SD	14.7	0.160	0.0591
2M	Mean	344.3	1.911	0.5556
	SD	22.0	0.155	0.0366
3M	Mean	377.0	1.897	0.5040
	SD	16.2	0.053	0.0294
4M	Mean	224.3	2.194	0.9832
	SD	9.4	0.418	0.2143

Group and sex		Body weight (g)	Lung weight (g)	Ratio (%)
1F	Mean	217.5	1.405	0.6461
	SD	17.4	0.131	0.0378
2F	Mean	237.4	1.502	0.6346
	SD	17.1	0.152	0.0733
3F	Mean	237.9	1.462	0.6148
	SD	25.1	0.154	0.0251
4F	Mean	180.5	1.778	0.9848
	SD	7.6	0.241	0.1272

8 APPENDICES

APPENDIX 1

Individual exposure chamber conditions

Group		Chamber condition in hour:								
		0	½	1	1½	2	2½	3	3½	4
1	Temperature °C	21	21	21	21	21	20	22	21	21
	Relative humidity %	50	52	52	50	50	50	50	50	50
	Air flow L/min	30	30	30	30	30	30	30	30	30
	Oxygen concentration %	20.8	20.9	20.9	21.1	21.0	20.9	21.0	21.0	20.8
2	Temperature °C	20	20	20	20	21	20	21	21	21
	Relative humidity %	40	35	35	32	30	30	30	30	30
	Air flow L/min	30	30	30	30	30	30	30	30	30
	Oxygen concentration %	20.7	20.5	20.5	20.6	20.5	20.4	20.4	20.4	20.4
3	Temperature °C	20	21	21	21	21	21	21	21	20
	Relative humidity %	65	35	30	30	30	29	29	29	29
	Air flow L/min	30	30	30	30	30	30	30	30	30
	Oxygen concentration %	20.7	20.7	20.7	20.7	20.6	20.6	20.6	20.6	20.6
4	Temperature °C	18	19	20	20	22	22	22	20	+
	Relative humidity %	45	30	28	28	28	28	28	26	+
	Air flow L/min	30	30	30	30	30	30	30	30	+
	Oxygen concentration %	20.8	21.0	21.0	21.0	21.0	21.0	20.9	21.0	+

+ exposure terminated before the end of the 4-hour period

APPENDIX 2

Individual and group mean body weights (g)

Group and sex	Animal number	Body weight (g) on Day: 1 Pre-exposure	1 Post-exposure	2	8	15
1M	1	237	226	241	296	341
	2	248	241	254	295	346
	3	244	231	242	306	360
	4	242	233	243	302	345
	5	245	235	241	287	319
	Mean	243	233	244	297	342
	SD	4	5	6	7	15
2M	21	288	268	277	314	350
	22	291	272	273	316	360
	23	270	249	240	281	316
	24	306	278	301	341	378
	25	297	277	273	313	358
	Mean	290	269	273	313	352
	SD	13	12	22	21	23
3M	31	329	309	310	344	369
	32	333	312	303	345	380
	33	340	317	313	350	381
	34	336	306	301	340	365
	35	375	341	327	365	410
	Mean	343	317	311	349	381
	SD	19	14	10	10	18
4M	11	247	Dead			
	12	254	Dead			
	13	231	Dead			
	14	229	Dead			
	15	250	Dead			
	Mean	242				
	SD	11				

Group and sex	Animal number	Body weight (g) on Day: 1 Pre-exposure	1 Post-exposure	2	8	15
1F	6	188	182	194	217	242
	7	205	190	193	220	232
	8	176	170	174	191	210
	9	188	181	188	207	226
	10	165	161	171	186	199
	Mean	184	177	184	204	222
	SD	15	11	11	15	17
2F	26	206	191	189	209	230
	27	212	199	201	224	240
	28	212	193	185	225	248
	29	198	185	196	212	230
	30	235	217	226	245	274
	Mean	213	197	199	223	244
	SD	14	12	16	14	18
3F	36	245	228	210	243	260
	37	246	221	209	230	252
	38	242	227	218	238	262
	39	208	199	202	212	224
	40	203	194	180	195	204
	Mean	229	214	204	224	240
	SD	21	16	14	20	25
4F	16	186	Dead			
	17	194	Dead			
	18	187	Dead			
	19	196	Dead			
	20	200	Dead			
	Mean	193				
	SD	6				

APPENDIX 3

Individual and group mean lung weights (g) and lung/body weight ratios (%)

Group and sex	Animal number	Body weight (g)	Lung weight (g)	Ratio (%)
1M	1	334.8	2.045	0.6108
	2	339.1	1.926	0.5680
	3	356.4	1.649	0.4627
	4	340.5	1.714	0.5034
	5	315.5	1.799	0.5702
	Mean	337.3	1.827	0.5430
	SD	14.7	0.160	0.0591
2M	21	346.4	1.863	0.5378
	22	351.8	2.163	0.6148
	23	307.7	1.742	0.5661
	24	367.3	1.920	0.5227
	25	348.2	1.869	0.5368
	Mean	344.3	1.911	0.5556
	SD	22.0	0.155	0.0366
3M	31	364.1	1.988	0.5460
	32	376.7	1.862	0.4942
	33	380.4	1.862	0.4895
	34	361.5	1.879	0.5198
	35	402.2	1.892	0.4704
	Mean	377.0	1.897	0.5040
	SD	16.2	0.053	0.0294
4M	11	227.9	2.365	1.0377
	12	237.2	2.000	0.8432
	13	215.0	2.570	1.1953
	14	215.2	2.480	1.1524
	15	226.2	1.555	0.6874
	Mean	224.3	2.194	0.9832
	SD	9.4	0.418	0.2143

Group and sex	Animal number	Body weight (g)	Lung weight (g)	Ratio (%)
1F	6	237.5	1.405	0.5916
	7	228.0	1.556	0.6825
	8	204.4	1.287	0.6296
	9	222.6	1.514	0.6801
	10	195.2	1.262	0.6465
	Mean	217.5	1.405	0.6461
	SD	17.4	0.131	0.0378
2F	26	226.1	1.519	0.6718
	27	234.6	1.236	0.5269
	28	239.7	1.561	0.6512
	29	221.3	1.593	0.7198
	30	265.1	1.599	0.6032
	Mean	237.4	1.502	0.6346
	SD	17.1	0.152	0.0733
3F	36	256.0	1.603	0.6262
	37	252.2	1.570	0.6225
	38	259.2	1.507	0.5814
	39	218.1	1.408	0.6456
	40	204.0	1.221	0.5985
	Mean	237.9	1.462	0.6148
	SD	25.1	0.154	0.0251
4F	16	175.1	1.977	1.1291
	17	184.2	2.048	1.1118
	18	170.4	1.482	0.8697
	19	183.2	1.599	0.8728
	20	189.5	1.782	0.9404
	Mean	180.5	1.778	0.9848
	SD	7.6	0.241	0.1272

APPENDIX 4

Individual necropsy findings - Group 1

Necropsy finding	Animal number and sex:									
	1M	2M	3M	4M	5M	6F	7F	8F	9F	10F
Animal not remarkable (NR)	NR		NR	NR	NR	NR	NR	NR	NR	NR
Tail: kinked, near tip		P								

Individual necropsy findings - Group 2

Necropsy finding	Animal number and sex:									
	21M	22M	23M	24M	25M	26F	27F	28F	29F	30F
Animal not remarkable (NR)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

Key: P = finding present, 1 - 5 = finding present and graded minimal - severe.

APPENDIX 4

Individual necropsy findings - Group 3

Necropsy finding	Animal number and sex:									
	31M	32M	33M	34M	35M	36F	37F	38F	39F	40F
Animal not remarkable (NR)		NR	NR	NR	NR			NR	NR	NR
Testis: small, right	3									
Mandibular lymph node: red						3				
Skin subcutis: fur loss, head							2			

Individual necropsy findings - Group 4

Necropsy finding	Animal number and sex:									
	11M	12M	13M	14M	15M	16F	17F	18F	19F	20F
Animal not remarkable (NR)										
Lung: dark, all lobes	4		5	4		4	4			
Lung: red, all lobes		3			2			2	2	3
Kidney: pelvic dilatation, left			2							
Stomach: distension			4							

Key: P = finding present, 1 - 5 = finding present and graded minimal - severe.

APPENDIX 5
SPONSOR'S CERTIFICATE OF ANALYSIS

HWI 6592-100

Certificate of Analysis

Test Material:	Imidan 70-WP, as used for HE Study Number 1316/1
Date of Analysis:	12 and 13 August 1994
Average % active ingredient:	70.2% w/w

Signature

September 20, 1994
Date

APPENDIX 6
PROBIT ANALYSIS

PROBIT ANALYSIS FOR PROJECT NUMBER 1316/1

1. SEXES COMBINED
==========

DOSE (MG/L)
0. 1.06000 2.08000 4.77000

GROUP SIZE
10. 10. 10. 10.

RESPONSE (DEATHS)
0. 0. 0. 10.

DOSE TRANSFORMED TO LOGARITHMS
999.000 0.025306 0.318063 0.678518
NOTE: ZERO DOSE IGNORED IN FIT

RESPONSE RATIOS (OBSERVED)
999. 0. 0. 1.00

WARNING - ONLY ZERO OR 100% RESPONSES

RESPONSE RATIOS (FITTED)
999. 0.000 0.000 1.000

FITTED PROBIT VALUES
999. -11.0 -4.23 4.16

PARAMETERS FOR THE PROBIT LINE Y=ALPHA + BETA*LOG(DOSE) FOLLOW

		S.E.
INTERCEPT (ALPHA)=	-11.6304	[26.5438]
SLOPE (BETA)=	23.2794	[48.2786]

CHISQUARED= 0.0003 DEVIANCE= 0.0005 (1. DF)
CHISQUARED NOT SIGNIFICANT AT THE SPECIFIED LEVEL OF 5.%
WARNING - SMALL EXPECTED RESPONSE RENDERS CHISQUARED UNRELIABLE

THE DATA DOES NOT ESTABLISH THE EXISTENCE OF A LINEAR PROBIT
RELATIONSHIP AT THE 5.% SIGNIFICANCE LEVEL
95.% FIDUCIAL LIMITS CANNOT BE CALCULATED

ED 50.00

ED 50.00= 0.499601 [S.E.= 0.373455]

DETRANSFORMED FROM LOGARITHMS:
ED 50.00= 3.15938

```
PROBIT ANALYSIS FOR PROJECT NUMBER 1316/1

2. MALES ONLY
=============

DOSE (MG/L)
  0.          1.06000    2.08000    4.77000

GROUP SIZE
        5.         5.         5.         5.

RESPONSE (DEATHS)
        0.         0.         0.         5.

DOSE TRANSFORMED TO LOGARITHMS
999.000  0.025306  0.318063  0.678518
NOTE: ZERO DOSE IGNORED IN FIT

RESPONSE RATIOS (OBSERVED)
   999.  0.      0.       1.00

WARNING - ONLY ZERO OR 100% RESPONSES

RESPONSE RATIOS (FITTED)
   999.  0.000  0.000   1.000

FITTED PROBIT VALUES
   999.  -10.9  -4.16   4.10


PARAMETERS FOR THE PROBIT LINE  Y=ALPHA + BETA*LOG(DOSE) FOLLOW
                                      S.E.
INTERCEPT (ALPHA)=   -11.4415   [   32.8178 ]
     SLOPE (BETA)=    22.9007   [   59.7424 ]

CHISQUARED=   0.0002    DEVIANCE=   0.0004 (     1. DF)
CHISQUARED NOT SIGNIFICANT AT THE SPECIFIED LEVEL OF    5.%
WARNING - SMALL EXPECTED RESPONSE RENDERS CHISQUARED UNRELIABLE

 THE DATA DOES NOT ESTABLISH THE EXISTENCE OF A LINEAR PROBIT
 RELATIONSHIP AT THE    5.% SIGNIFICANCE LEVEL
   95.% FIDUCIAL LIMITS CANNOT BE CALCULATED


ED   50.00
----------
ED   50.00=  0.499614   [S.E.=  0.469780]

DETRANSFORMED FROM LOGARITHMS:
ED   50.00=   3.15947
```

PROBIT ANALYSIS FOR PROJECT NUMBER 1316/1

3. FEMALES ONLY
==========

DOSE (MG/L)
0. 1.06000 2.08000 4.77000

GROUP SIZE
5. 5. 5. 5.

RESPONSE (DEATHS)
0. 0. 0. 5.

DOSE TRANSFORMED TO LOGARITHMS
999.000 0.025306 0.318063 0.678518
NOTE: ZERO DOSE IGNORED IN FIT

RESPONSE RATIOS (OBSERVED)
999. 0. 0. 1.00

WARNING - ONLY ZERO OR 100% RESPONSES

RESPONSE RATIOS (FITTED)
999. 0.000 0.000 1.000

FITTED PROBIT VALUES
999. -10.9 -4.16 4.10

PARAMETERS FOR THE PROBIT LINE Y=ALPHA + BETA*LOG(DOSE) FOLLOW

		S.E.
INTERCEPT (ALPHA)=	-11.4415	[32.8175]
SLOPE (BETA)=	22.9007	[59.7424]

CHISQUARED= 0.0002 DEVIANCE= 0.0004 (1. DF)
CHISQUARED NOT SIGNIFICANT AT THE SPECIFIED LEVEL OF 5.%
WARNING - SMALL EXPECTED RESPONSE RENDERS CHISQUARED UNRELIABLE

THE DATA DOES NOT ESTABLISH THE EXISTENCE OF A LINEAR PROBIT
RELATIONSHIP AT THE 5.% SIGNIFICANCE LEVEL
95.% FIDUCIAL LIMITS CANNOT BE CALCULATED

ED 50.00

ED 50.00= 0.499614 [S.E.= 0.469780]

DETRANSFORMED FROM LOGARITHMS:
ED 50.00= 3.15947

```
PROBIT ANALYSIS FOR PROJECT NUMBER 1316/1

1. SEXES COMBINED
==================

DOSE (MG/L)
  0.        0.660000  0.610000   3.69000

GROUP SIZE
      10.       10.       10.       10.

RESPONSE (DEATHS)
       0.        0.        0.       10.

DOSE TRANSFORMED TO LOGARITHMS
999.000 -0.180456 -0.214670  0.567026
NOTE: ZERO DOSE IGNORED IN FIT

RESPONSE RATIOS (OBSERVED)
   999.  0.     0.      1.00

WARNING - ONLY ZERO OR 100% RESPONSES

RESPONSE RATIOS (FITTED)
   999.  0.000  0.000   1.000

FITTED PROBIT VALUES
   999.  -4.56  -4.97   4.30


PARAMETERS FOR THE PROBIT LINE  Y=ALPHA + BETA*LOG(DOSE) FOLLOW
                                      S.E.
INTERCEPT (ALPHA)=   -2.42197   [   18.7603 ]
     SLOPE (BETA)=    11.8670   [   38.2604 ]

CHISQUARED=   0.0001    DEVIANCE=   0.0002 (    1. DF)
CHISQUARED NOT SIGNIFICANT AT THE SPECIFIED LEVEL OF    5.%
WARNING - SMALL EXPECTED RESPONSE RENDERS CHISQUARED UNRELIABLE

 THE DATA DOES NOT ESTABLISH THE EXISTENCE OF A LINEAR PROBIT
 RELATIONSHIP AT THE    5.% SIGNIFICANCE LEVEL
   95.% FIDUCIAL LIMITS CANNOT BE CALCULATED


ED   50.00
----------
ED   50.00=  0.204094   [S.E.=   1.19560]

DETRANSFORMED FROM LOGARITHMS:
ED   50.00=   1.59990
```

```
PROBIT ANALYSIS FOR PROJECT NUMBER 1316/1

2. MALES ONLY
=============

DOSE (MG/L)
  0.         3.660000  0.610000   3.69000

GROUP SIZE
        5.         5.         5.         5.

RESPONSE (DEATHS)
        0.         0.         0.         5.

DOSE TRANSFORMED TO LOGARITHMS
999.000 -0.180456 -0.214670  0.567026
NOTE: ZERO DOSE IGNORED IN FIT

RESPONSE RATIOS (OBSERVED)
   999.  0.      0.      1.00

WARNING - ONLY ZERO OR 100% RESPONSES

RESPONSE RATIOS (FITTED)
   999.  0.000  0.000  1.000

FITTED PROBIT VALUES
   999.  -4.45  -4.85   4.20



PARAMETERS FOR THE PROBIT LINE  Y=ALPHA + BETA*LOG(DOSE) FOLLOW
                                          S.E.
INTERCEPT (ALPHA)=   -2.36302   [   20.7887 ]
     SLOPE (BETA)=    11.5762   [   42.7365 ]

CHISQUARED=   0.0001    DEVIANCE=   0.0002 (    1. DF)
CHISQUARED NOT SIGNIFICANT AT THE SPECIFIED LEVEL OF    5.%
WARNING - SMALL EXPECTED RESPONSE RENDERS CHISQUARED UNRELIABLE

 THE DATA DOES NOT ESTABLISH THE EXISTENCE OF A LINEAR PROBIT
 RELATIONSHIP AT THE    5.% SIGNIFICANCE LEVEL
   95.% FIDUCIAL LIMITS CANNOT BE CALCULATED



ED   50.00
----------
ED   50.00=  0.204128   [S.E.=   1.37086]

DETRANSFORMED FROM LOGARITHMS:
ED   50.00=   1.60003
```

PROBIT ANALYSIS FOR PROJECT NUMBER I316/1

3. FEMALES ONLY
==========

DOSE (MG/L)
0. 0.660000 0.610000 3.69000

GROUP SIZE
5. 5. 5. 5.

RESPONSE (DEATHS)
0. 0. 0. 5.

DOSE TRANSFORMED TO LOGARITHMS
999.000 -0.180456 -0.214670 0.567026
NOTE: ZERO DOSE IGNORED IN FIT

RESPONSE RATIOS (OBSERVED)
999. 0. 0. 1.00

WARNING - ONLY ZERO OR 100% RESPONSES

RESPONSE RATIOS (FITTED)
999. 0.000 0.000 1.000

FITTED PROBIT VALUES
999. -4.45 -4.85 4.20

PARAMETERS FOR THE PROBIT LINE Y=ALPHA + BETA*LOG(DOSE) FOLLOW

		S.E.
INTERCEPT (ALPHA)=	-2.36302	[20.7887]
SLOPE (BETA)=	11.5762	[42.7365]

CHISQUARED= 0.0001 DEVIANCE= 0.0002 (1. DF)
CHISQUARED NOT SIGNIFICANT AT THE SPECIFIED LEVEL OF 5.%
WARNING - SMALL EXPECTED RESPONSE RENDERS CHISQUARED UNRELIABLE

THE DATA DOES NOT ESTABLISH THE EXISTENCE OF A LINEAR PROBIT
RELATIONSHIP AT THE 5.% SIGNIFICANCE LEVEL
95.% FIDUCIAL LIMITS CANNOT BE CALCULATED

ED 50.00

ED 50.00= 0.204128 [S.E.= 1.37086]

DETRANSFORMED FROM LOGARITHMS:
ED 50.00= 1.60003

PROBIT ANALYSIS FOR PROJECT NUMBER 1316/1

1. SEXES COMBINED
==========

DOSE (MG/L)
0. 0.460000 0.430000 2.59000

GROUP SIZE
10. 10. 10. 10.

RESPONSE (DEATHS)
0. 0. 0. 10.

DOSE TRANSFORMED TO LOGARITHMS
999.000 -0.337242 -0.366532 0.413300
NOTE: ZERO DOSE IGNORED IN FIT

RESPONSE RATIOS (OBSERVED)
999. 0. 0. 1.00

WARNING - ONLY ZERO OR 100% RESPONSES

RESPONSE RATIOS (FITTED)
999. 0.000 0.000 1.000

FITTED PROBIT VALUES
999. -4.58 -4.92 4.30

PARAMETERS FOR THE PROBIT LINE Y=ALPHA + BETA*LOG(DOSE) FOLLOW

		S.E.
INTERCEPT (ALPHA)=	-0.584091	[15.0969]
SLOPE (BETA)=	11.8354	[38.2368]

CHISQUARED= 0.0001 DEVIANCE= 0.0002 (1. DF)
CHISQUARED NOT SIGNIFICANT AT THE SPECIFIED LEVEL OF 5.%
WARNING - SMALL EXPECTED RESPONSE RENDERS CHISQUARED UNRELIABLE

THE DATA DOES NOT ESTABLISH THE EXISTENCE OF A LINEAR PROBIT
RELATIONSHIP AT THE 5.% SIGNIFICANCE LEVEL
95.% FIDUCIAL LIMITS CANNOT BE CALCULATED

ED 50.00

ED 50.00= 0.049351 [S.E.= 1.20242]

DETRANSFORMED FROM LOGARITHMS:
ED 50.00= 1.12034

PROBIT ANALYSIS FOR PROJECT NUMBER 1316/1

2. MALES ONLY
==========

DOSE (MG/L)
0. 0.460000 0.430000 2.59000

GROUP SIZE
5. 5. 5. 5.

RESPONSE (DEATHS)
0. 0. 0. 5.

DOSE TRANSFORMED TO LOGARITHMS
999.000 -0.337242 -0.366532 0.413300
NOTE: ZERO DOSE IGNORED IN FIT

RESPONSE RATIOS (OBSERVED)
999. 0. 0. 1.00

WARNING - ONLY ZERO OR 100% RESPONSES

RESPONSE RATIOS (FITTED)
999. 0.000 0.000 1.000

FITTED PROBIT VALUES
999. -4.46 -4.80 4.20

PARAMETERS FOR THE PROBIT LINE Y=ALPHA + BETA*LOG(DOSE) FOLLOW

		S.E.
INTERCEPT (ALPHA)=	-0.570482	[16.8202]
SLOPE (BETA)=	11.5460	[42.6900]

CHISQUARED= 0.0001 DEVIANCE= 0.0002 (1. DF)
CHISQUARED NOT SIGNIFICANT AT THE SPECIFIED LEVEL OF 5.%
WARNING - SMALL EXPECTED RESPONSE RENDERS CHISQUARED UNRELIABLE

THE DATA DOES NOT ESTABLISH THE EXISTENCE OF A LINEAR PROBIT
RELATIONSHIP AT THE 5.% SIGNIFICANCE LEVEL
95.% FIDUCIAL LIMITS CANNOT BE CALCULATED

ED 50.00

ED 50.00= 0.049409 [S.E.= 1.37791]

DETRANSFORMED FROM LOGARITHMS:
ED 50.00= 1.12049

PROBIT ANALYSIS FOR PROJECT NUMBER 1316/1

3. FEMALES ONLY
==========

DOSE (MG/L)
0. 0.460000 0.430000 2.59000

GROUP SIZE
5. 5. 5. 5.

RESPONSE (DEATHS)
0. 0. 0. 5.

DOSE TRANSFORMED TO LOGARITHMS
999.000 -0.337242 -0.366532 0.413300
NOTE: ZERO DOSE IGNORED IN FIT

RESPONSE RATIOS (OBSERVED)
999. 0. 0. 1.00

WARNING - ONLY ZERO OR 100% RESPONSES

RESPONSE RATIOS (FITTED)
999. 0.000 0.000 1.000

FITTED PROBIT VALUES
999. -4.46 -4.80 4.20

PARAMETERS FOR THE PROBIT LINE Y=ALPHA + BETA*LOG(DOSE) FOLLOW

		S.E.
INTERCEPT (ALPHA)=	-0.570482	[16.8202]
SLOPE (BETA)=	11.5460	[42.6900]

CHISQUARED= 0.0001 DEVIANCE= 0.0002 (1. DF)
CHISQUARED NOT SIGNIFICANT AT THE SPECIFIED LEVEL OF 5.%
WARNING - SMALL EXPECTED RESPONSE RENDERS CHISQUARED UNRELIABLE

THE DATA DOES NOT ESTABLISH THE EXISTENCE OF A LINEAR PROBIT RELATIONSHIP AT THE 5.% SIGNIFICANCE LEVEL
95.% FIDUCIAL LIMITS CANNOT BE CALCULATED

ED 50.00

ED 50.00= 0.049409 [S.E.= 1.37791]

DETRANSFORMED FROM LOGARITHMS:
ED 50.00= 1.12049

HAZLETON EUROPE

APPENDIX 7

ANALYTICAL PROCEDURE

HE 1316/1-02F

Determination of Phosmet in

dosing formulations of Imidan 70-WP

Effective date: 20 October 1994

Written by: M. [illegible]

Authorised by: [illegible]

Determination of Phosmet in dosing formulations of Imidan 70-WP

1 GENERAL PRINCIPLES

Samples of formulation are diluted with acetone and are analysed using gas chromatography (GC). Concentrations of test article are calculated from standard solutions in a calibration line.

2 SAFETY AND HANDLING

All procedures in this method have handling and control codes which are detailed in Appendix 1.

Laboratory coats and safety glasses (categories 2a and 3a) must be worn at all times in the laboratory.

The test article should only be used in a fume cupboard. Avoid contact with skin, eyes and clothing. Do not breath vapours. Operators should take the normal precautions of wearing gloves when handling it.

3 APPARATUS, MATERIALS, REAGENTS AND SOLUTIONS

3.1 Apparatus, glassware etc

General laboratory glassware, including volumetric flasks, etc
Electronic digital pipette (EDP)
Autosampler vials and caps

3.2 Materials

The materials listed below should be labelled with a hazard symbol or other marking to indicate their potential hazards. The hazard should be listed below if this is not the case. If there is no marking or indication consult the responsible scientist.

Acetone	- Glass distilled - Rathburn Chemicals Limited
Analytical Standard	- Phosmet, a colourless crystalline solid - Chem Service, Inc, - supplied with Certificate of Analysis
Internal standard	- 9-Phenylcarbazole, a white powder - Aldrich Chemicals

3.3 Reagents and Solutions

3.3.1 Test article fortifying solution

Analytical standard (approximately 0.42 mg) is accurately weighed. [1a, 4c]

It is then dissolved in acetone and made to 10 mL. [1a, 4b]

Two solutions (A and B) of approximately equal concentrations are prepared.

3.3.2 Internal standard solution

Internal standard (approximately 4.0 mg) is accurately weighed. [1a, 4c]

It is then dissolved in acetone and made to 10 mL. [1a, 4b]

4.1 Generation of calibration line

Test article fortifying solution A (0.2, 0.6, 1.0 mL) and test article fortifying solution B (0.4, 0.8, 1.2 mL) are added to respective volumetric flasks (10 mL) together with 1 mL of internal standard solution. They are made to volume with acetone. [1a, 4a]

4.2 Extraction of samples

The formulations are diluted down to within the range of the calibration line (0 to 5 µg/mL) using acetone. The final dilution must be 1 mL to 10 mL into a volumetric flask containing 1 mL of internal standard. [1a, 4a]

The final sample and calibration solutions are then submitted to GC under the conditions specified below in Section 4.3. [4a]

4.3 Gas chromatography

Column	- J+W Scientific DB1, 15m x 0.53mm ID, 1.5 µm film thickness
Oven	- Isothermal, 240°C
Injection	- Autosampler with split injection. Injection volume of 5 µL. Injection port at 240°C
Carrier gas	- Helium at 10 psi
Detector	- N/P detector at 300°C
Detector gases	- Hydrogen, flow rate 3.5 mL/min Air, flow rate 150 mL/min

Internal standard is retained for aproximately 1.5 minutes, Phosmet for 1.9 minutes. See specimen chromatogram in Appendix 2.

5 CALCULATIONS

The peak area ratio of the calibration standard solutions/internal standard solutions (Y) and the amount of test article (X) are used to generate a straight line graph ($Y = MX + C$) where C is the constant and M is the 1st degree.

Concentration of test article (X) in the sample is calculated from the resulting equation:

All peak area measurements and calculations are performed on a Multichrom data capture system using software version 2.0.

$$X = \frac{(Y - C)}{M}$$

6 DEVIATIONS FROM THIS METHOD

Any minor practical changes to this written procedure which may be necessary are recorded, along with results, calibration and chromatogram data.

7 COSHH ASSESSMENT OF THIS METHOD

The hazards and risks of the substances used in this method have been assessed. There should be no foreseeable hazards to health, provided that the method is accurately followed and the control measures specified in the method are correctly used.

Page: 6 of 7
Analytical Procedure: HE 1316/1-02F
Test Article: Imidan 70-WP
Issue Date: 20 October 1994

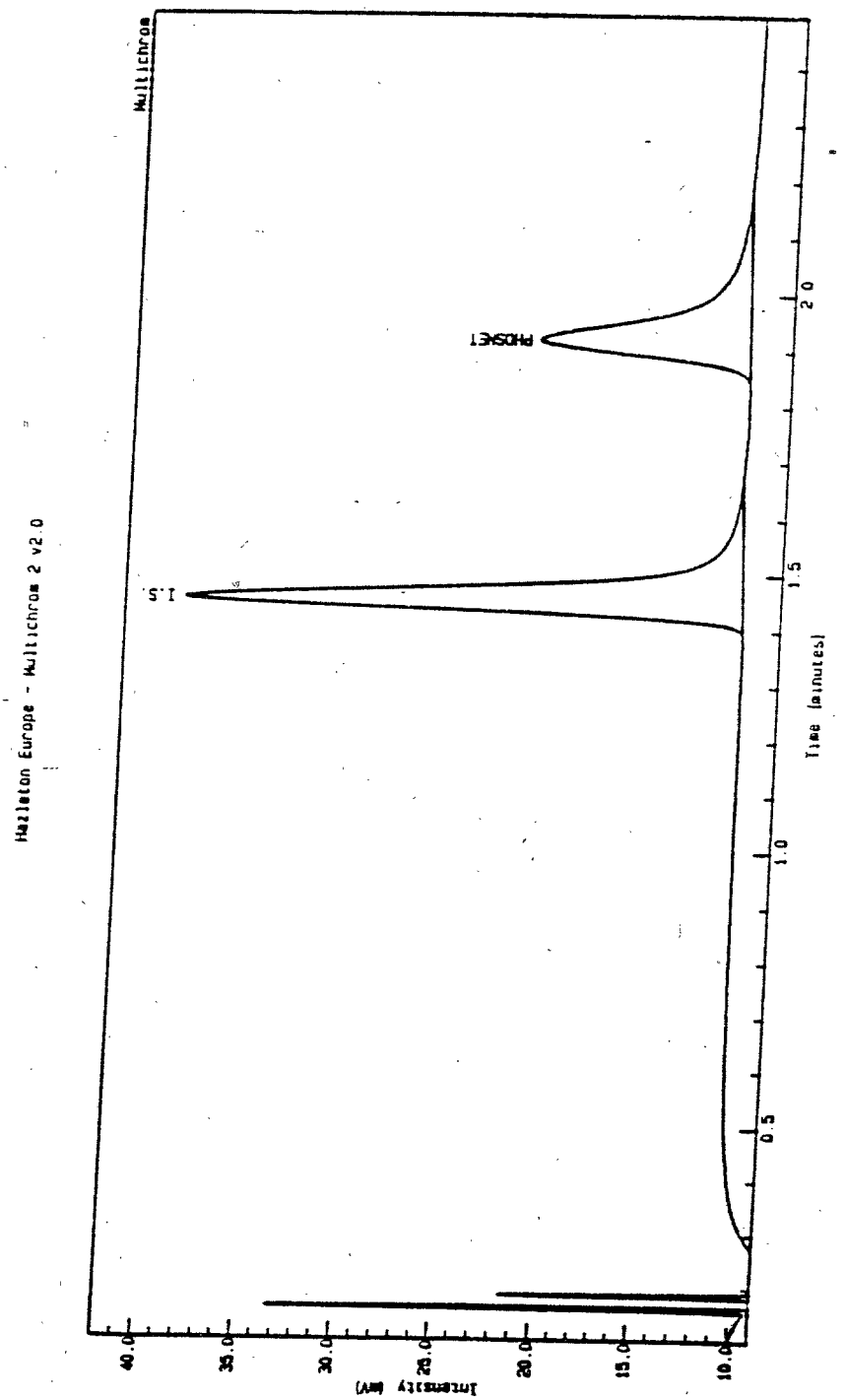
APPENDIX 1

General Handling Control Categories

CATEGORY Main	Division	CONTROL Name and Specification
1		**GLOVES**
	a	Disposable latex
	b	Disposable Nitrile
	c	Rubber gloves
	d	Specific type for job (see assessment giving details)
2		**PROTECTIVE CLOTHING**
	a	Laboratory coat or equivalent
	b	Disposable overalls
	c	Oversleeves
	d	Overshoes
	e	Plastic apron
	f	Protective footwear (give details)
3		**EYE/FACE PROTECTION**
	a	Safety glasses to BS 2092/2 or better
	b	Face shield to BS 2092/2 C or better
	c	Safety Goggles to BS 2092/2 C or better
4		**ENGINEERING CONTROLS**
	a	Open bench in ventilated area
	b	Fume cupboard to BS 7258
	c	Laminar flow cabinet to BS 5295 Class 1
	d	Re-circulating fume chamber
	e	Radioisotope laboratory
	f	Biohazard laboratory
	g	Shield and isolate (explosive)
	h	Plymouth extractor LF400
	i	Glove Box
5		**RESPIRATORY PROTECTIVE EQUIPMENT**
	a	Disposable filtering facemask (HSE approved) • organic vapour • dust • combination organic vapour/dust MUST SPECIFY TYPE
	b	Powered respirators/helmets with safety visor to BS 2092/2 C or better (HSE approved)
	c	Respirator with specified canister (HSE approved)
	d	Full facemask supplied with breathing quality air (HSE approved)
6		**SPECIFIC IMMUNISATION REQUIRED** (GIVE DETAILS)
7		**ALLERGIC PERSONS PROHIBITED** (SPECIFY ALLERGY)
8		**REFER TO MATERIAL SAFETY DATA SHEET**
9		**KNOWN OR SUSPECTED REPRODUCTIVE HAZARD** TO EITHER SEX (must specify details).
10		**POISON** - ensure antidote is available and is within its expiry date (must specify details)

APPENDIX 2

Specimen Chromatogram



APPENDIX 8
STUDY PROTOCOL

HAZLETON
EUROPE
Otley Road, Harrogate
North Yorkshire HG3 1PY England

PROTOCOL APPROVAL PAGE

Study Title: IMIDAN 70-WP: SINGLE DOSE INHALATION TOXICITY STUDY IN RATS

Regulatory (Test) Guidelines: Protocol is designed to meet the known requirements of EPA Guideline 81-3

HE Study Number: 1316/1

HE Protocol Number: P9368d

HE Study Director A P Mould Date 2 June 1994

Name A P Mould

HE Management C J Collins Date 2 June 1994

Name, Title C J Collins, Section Manager - Inhalation

Authorised by Sponsor** Bethany G Hulcy Date 6 June 1994

Name, Title (Print or type) Registration Specialist

Sponsor's Monitor: Bethany Hulcy

Sponsor: Gowan Company
Box 5569
Yuma
Arizona 85366-5569
USA

** Please print or type your name and Company status below your signature and return this page to the Study Director

1. OBJECTIVE

To estimate the approximate 4 hour acute inhalation median lethal concentration of the test article, Imidan 70-WP, in the rat.

The test article will be assigned a toxicity category according to the toxicity assessment criteria (Appendix 5). This determination of toxicity class minimises the animal usage. The data obtained may not permit computation of the acute median lethal concentration but where possible this will be estimated.

2. EXPERIMENTAL DESIGN

: prior to the main study, 1 male and 1 female may be exposed for up to 4 hours at the maximum easily attainable concentration, in order to set the dose levels for the main study. This concentration would normally be not less than approximately 1 mg/L and could be as high as 5 mg/L

Limit test : for a material of unknown inhalation toxicity an initial exposure of 5 males and 5 females will be conducted at 5 mg/L, or the maximum achievable concentration if this is less than 5 mg/L. A control group will be exposed to chamber air only

Definitive study : if deaths occur at levels of up to 5 mg/L a definitive study will normally be performed

incorporating the test and control groups already exposed

Group number	Animals/group		Dose levels mg/L
	Male	Female	
1	5	5	0 (air control)
2	5	5	
3	5	5	
4	5	5	

The animals will be necropsied at Day 15

: exposures will be conducted sequentially. The number of dose levels may be varied at the discretion of the study director in order to achieve the objectives of the study

3. TEST SYSTEM

Species : rat of the Crl:CD(SD)BR strain

Supplier : Charles River (UK) Ltd

Weight on arrival : preferably less than 7 weeks but not more than 8 weeks old at start of dosing, weight range ± 20% of the overall mean for each sex at allocation

Number/sex : 5 male, 5 female per group

Justification : one of the rodent species recommended by various regulatory authorities.

Background data are available on the selected strain

Animal health and welfare : all procedures to be carried out on live animals as part of this study will be subject to the provisions of United Kingdom National Law, in particular the Animals (Scientific Procedures) Act, 1986

4. ENVIRONMENT AND HUSBANDRY

Air conditioning : minimum 15 air changes/hour

Temperature : 19 to 25°C

Humidity : 40 to 70%

Lighting : 12 hours light (0600 to 1800) /12 hours dark

Caging : suspended stainless steel mesh cages (5 animals/cage)

5. DIET AND WATER

Diet : SQC Rat and Mouse Maintenance Diet No 1 Expanded, Special Diets Services Ltd ad libitum except during exposure. Each batch of diet is analysed for specific contaminants. Typical values are presented in Appendix 1

Water	: mains water ad libitum except during exposure. The water is periodically analysed for specific contaminants. Typical values are presented in Appendix 2
Contaminants	: no contaminants are expected to be present in the diet or water at levels which might interfere with achieving the objectives of the study

6. PRE-EXPERIMENTAL PROCEDURES

Animal health procedures	: all animals will be given a clinical inspection for ill-health on arrival. Clinical inspection will be performed before start of dosing
Acclimatisation period	: minimum 5 days
Allocation to treatment group	: total randomisation on arrival, arbitrary allocation by cage to study
Identification	: indelible ink marking on tail
	: a colour-coded card on each cage will give information including study number and animal numbers

7. TEST ARTICLE

Test article	: Imidan 70-WP, supplied as a powder. Prior to use, the test article will be micronised by Micron Mills Limited, Kent, UK, who operate under full GMP approval by the US FDA, and hold a Medicines Control Agency licence. Hazleton dispensary number to be allocated
Specification	: supplied by sponsor
Hazardous properties	: HE routine handling procedures sufficient
Storage of test article	: room temperature in a desiccator
Control article	: filtered air
Formulation	: as supplied
Administration	: inhalation - head-only on an aluminium chamber of appropriate size and internal volume for the exposure regimen (Appendix 3)
Justification	: possible route of human exposure
Frequency/duration of administration	: single exposure of 4 hours
Generation	: respirable particles generated by standard methods from the bulk test article

Stability	: expiry date stated to be end of October 1996
Toxicokinetics	: not necessary to achieve objectives of study

8. ATMOSPHERE CONTROL

Temperature	: monitored continually and recorded twice hourly
	: target 20-24°C
Humidity	: monitored continually and recorded twice hourly
	: target 40-60%
Air flow	: not less than 12 air changes/hour, monitored continually and recorded twice hourly
Oxygen concentration	: measured continuously and recorded twice hourly
	: target at least 19%
Nominal concentration	: test article weighed before and after dosing to demonstrate correct delivery
Actual concentration	: measured at least hourly gravimetrically for the appropriate groups. The particulate trapped on the

filters will be analysed for Imidan

Particle size : measured prior to start of exposures and then hourly for each test group using gravimetric methodology. Mass mean aerodynamic diameter and geometric standard deviation calculated

: attempts will be made to achieve an atmosphere containing 25% by mass less than 1 μm mass median aerodynamic diameter in accordance with EPA requirements

Analysis of samples : the above sampling regimen will result routinely in the following number of batches of samples being submitted for analysis by gas chromatography. For each test group used concentration samples will be analysed in one batch. Prior method development will normally result in analysis on 5 occasions

9. EXPERIMENTAL OBSERVATIONS

Morbidity/mortality inspection : all animals will be observed twice daily at the beginning and end of each working day. Any animal which shows marked signs of ill-health will be isolated

and may be killed and necropsied to prevent cannibalism or autolysis

Clinical signs : hourly during exposure and for the remainder of the exposure day, then daily until termination. The study will be extended if significant abnormalities remain after 14 days

Body weights : on day of dosing before and after exposure and on days 2, 8 and 15 of the study

10. PATHOLOGY

Necropsy : necropsies will be performed on all animals that die or are killed during the course of the study and on all animals that survive to termination on Day 15. Animals will be weighed before necropsy

: animals will be killed by intraperitoneal pentobarbitone sodium. After exsanguination a full macroscopic examination will be performed

: nasal cavity and respiratory tract will be examined and any irritation assessed. Lungs and trachea will be weighed. All

gross lesions will be preserved in 10% neutral buffered formalin (except eyes - fixed in Davidson's fluid). Nasal cavity will not normally be examined histologically as the macroscopic examination precludes preservation

Histopathology : none in the first instance. If treatment-related lesions are identified macroscopically, the sponsor will be consulted to see if histopathology is required. If histopathology is required, a protocol amendment and cost estimate will be prepared by the Study Director

11. DATA EVALUATION : means and standard deviations calculated where appropriate Median lethal concentration (and 95% fiducial limits) calculated using a probit analysis method, where the data permits

12. GOOD LABORATORY PRACTICE COMPLIANCE : this study will be conducted in accordance with the UK Department of Health GLP Compliance Programme, 1989

: all procedures will be performed in accordance with detailed Standard Operating Procedures

: the records to be kept for this study are indicated in Appendix 4

: protocol and final report audits will be performed

: internal audits may be performed during the in-life

: wherever possible any change to this protocol will be made by an amendment agreed by Hazleton Europe and the study sponsor

13. REPORTS

: the sponsor will be informed promptly of any significant findings and issued a progress report at the end of the exposure period

: the final report will contain all procedures and results. An unaudited draft will be issued for discussion with the sponsor (see Appendix 6)

14. ARCHIVE

: all primary data, or authenticated copies thereof, specimens and the final report will be retained in the Hazleton Europe archives for three years after submission of the final report. At this time the

sponsor will be contacted to determine whether data should be returned, retained or destroyed on their behalf

June 1994

APPENDIX 1

DIET SUMMARY

Year ending 31 December 1993

R & M No 1 Expanded

Nutrients			Mean	SD
Moisture	%		9.6	0.5
Crude fat	%		3.2	0.1
Crude protein	%		15.5	0.7
Crude fibre	%		3.6	0.6
Ash	%		4.8	0.2
Calcium	%		0.75	0.09
Phosphorous	%		0.58	0.04
Sodium	%		0.25	0.02
Chloride	%		0.37	0.07
Potassium	%		0.73	0.03
Magnesium	%		0.15	0.01
Iron	mg/kg		167	32
Copper	mg/kg		11	2
Manganese	mg/kg		54	5
Zinc	mg/kg		40	6
Vitamin A	iu/g		4.6	0.6
Vitamin E	mg/kg		63	8
Vitamin C	mg/kg		-	-
Contaminants				
Fluoride	mg/kg		13	4
Nitrate as $NaNO_3$	mg/kg		26	10
Nitrite as $NaNO_2$	mg/kg		2.5	0.8
Lead	mg/kg		0.46	0.19
Arsenic	mg/kg	ND	-	
Cadmium	mg/kg		0.08	0.02
Mercury	mg/kg	ND	-	
Selenium	mg/kg		0.08	0.03
Total Aflatoxins	µg/kg	ND	-	
Total PCB	µg/kg	ND	-	
Total DDT	µg/kg		2	0
Dieldrin	µg/kg	ND	-	
Lindane	µg/kg		18	18
Heptachlor	µg/kg	ND	-	
Malathion	µg/kg		91	59
Total viable organisms x 10^3	per g		2.25	0.00
Mesophilic spores x 10^2	per g		4.50	2.81
Salmonella species	per g	ND	-	
Presumptive E coli	per g	ND	-	
E coli type 1	per g	ND	-	
Fungal units	per g		25	0
Antibiotic activity		ND	-	

ND = none detected

APPENDIX 2

Hazleton water supply - sampled at point of use (treated water)

Summary of water analysis January to December 1993

Parameter	Unit	Range	Mean
pH		7.40 - 8.52	7.85
Conductivity (20°C)	µs cm	123 - 143	131
Turbidity	F T U	0.1 - 6.2	0.4
Filtered colour	HAZEN	1.6 - 4.5	3.3
Nitrogen Ammoniac	N mg/L	<0.04	<0.04
Nitrogen Total Oxide	N mg/L	0.8 - 1.0	0.9
Nitrate	N mg/L	0.8 - 0.99	0.93
Nitrite	N mg/L	<0.003 - 0.011	<0.003
Hardness Total	$CaCO_3$ mg/L	45.53 - 49.59	46.12
Alkalinity Total	$CaCO_3$ mg/L	12 - 28	17
Chloride	Cl mg/L	12.5 - 17.0	14.8
Ortho-phosphate	P mg/L	<0.1	<0.1
Sulphate	SO_4 mg/L	26.6 - 30.6	28.8
Sodium Total	Na mg/L	7.9 - 9.2	8.52
Potassium Total	K mg/L	1.0 - 1.45	1.20
Copper Total	Cu mg/L	<0.01 - 0.166	<0.045
Magnesium Total	Mg mg/L	2.8 - 3.37	3.04
Calcium Total	Ca mg/L	13.3 - 14.4	13.9
Zinc Total	Zn mg/L	<0.01 - 6.07	<0.325
Aluminium Total	Al mg/L	<0.01 - 0.122	<0.067
Lead Total	Pb mg/L	<0.005 - 0.021	<0.006
Manganese Total	Mn mg/L	0.001 - 0.025	0.014
Iron Total	Fe mg/L	<0.01 - 0.107	<0.044
Aldrin	µg/L	<0.002 - <0.001	<0.001
HCH (BHC) ALPH	µg/L	<0.001 - 0.001	<0.001
HCH (BHC) GAMM	µg/L	<0.001 - 0.001	<0.001
Dieldrin	µg/L	<0.002 - 0.002	<0.002
DDE (PP)	µg/L	<0.01	0
DDT (PP)	µg/L	<0.005 - <0.01	<0.007
Endrin	µg/L	<0.01 - 0.04	<0.01
Chlorine Free	Cl mg/L	0.1 - 0.2	<0.12
Chlorine Total	Cl mg/L	0.1 - 0.2	<0.14
Colonies 3 day (22°C)	No/mL	1 - 5940	301
Colonies 1 day (37°C)	No/mL	1 - 275	7
Faecal Coliforms	No/100 mL	2	0
E Coli	No/100 mL	1	0
Total Coliforms	No/100 mL	2	0

APPENDIX 3

APPENDIX 4

1. STUDY RECORDS

The following study records will be maintained:

Study correspondence
Study file note(s)
Study log
Animal data file
Environmental records
Inhalation chemistry
Dispensary
Analytical chemistry
Necropsy
Histology (if any)
Statistics (if any)

APPENDIX 5

The data obtained in the single dose inhalation study can be compared with the toxicity classification below:

US EPA labelling requirements for Pesticides and Devices (four hour exposure) 40 CFR part 156.10.

Category	LC_{50} mg/L
I	<0.2
II	0.2 - 2.0
III	2.0 - 20.0
IV	>20.0

It should be noted that for labelling purposes, there is no difference between Toxicity Categories III and IV, ie the signal word on the front panel is identical.

Although the upper LC_{50} level for labelling purposes is 20 mg/L, if there are no deaths at or above 5 mg/L, then further single dose inhalation testing is not required by EPA. (Pesticide Assessment Guidelines, subdivision F, Addendum 6 on Acute and Subchronic Inhalation Toxicity Testing, October 1988).

APPENDIX 6

1. DRAFT FINAL REPORT

A complete unaudited draft report will be issued for the study sponsor's comments. The report will be prepared to contain the following information:

1.1 The objectives and procedures stated in the approved protocol including any changes made to the original protocol.

1.2 The identity of the test/control substances (by name or code number) and their strength (quality/purity).

1.3 The test system - species, strain and sex of the animals used.

1.4 Procedure for identification of the test system.

1.5 The dose levels used, the dosage regimen, route of administration and duration of treatment.

1.6 Any unforeseen circumstances which may have affected the quality or integrity of the study.

1.7 The reports of the individual scientists involved in the study, e.g. pathologist.

1.8 The location of all raw data and final report.

1.9 The name and address of the testing facility, start and completion dates of the study.

1.10 The following items of data will be presented:
experimental design
inhalation chemistry data
morbidity and mortality

clinical observations (toxic and pharmacological effects, condition and behaviour)

effects on body weight

lung weights and lung/body weight ratios

macroscopic pathology findings

LC_{50} value for sexes combined, and each sex if possible, determined at day 15 of the study. The method of calculation will be specified

2. FINAL REPORT

The final report will be issued following quality assurance evaluation of the complete draft report. This report will include all the details described in Section 1 above with the following additions:

2.1 The signature of the Study Director and other scientists involved in the study as authentication of the report.

2.2 A statement that the study and the report have been subject to quality assurance evaluation.

STUDY NO 1316/1

APPENDIX 7

RESPONSIBLE PERSONNEL

	NAME
Study Director[1]	A P Mould
Deputy Study Director	A C Gibbs
Head of Pathology	J R Glaister
Inhalation Chemist	B Canham
Formulations Analysis	I Fraser
Animal House Supervisor	J Cunningham (2 copies)
Animal Health and Welfare	A Basford
Necropsy	I Wilkins
Histology	S Brogden
Head of Quality Assurance	S White

PROPOSED DATES

Animals on site	June 1994
First treatment	June 1994
Study termination	July 1994
Draft report	August 1994

1 = Any change documented by protocol amendment
Any other change to personnel or dates documented in study records

DISTRIBUTION : Personnel above, Production Planning
Contracts Administration via Production Planning

DEVIATIONS FROM PROTOCOL

Section 4 Environment and husbandry

The temperature and relative humidity of the holding room were slightly outside the ranges specified in the protocol of 19 to 25°C and 40 to 70% respectively. The actual ranges recorded were 15 to 28°C and 34 to 74%.

Section 8 Chamber temperature

On two occasions during the exposure of the high dose group, the temperature in the exposure system fell below the limit set by the protocol. The actual lower limit was 18°C.

Section 8 Chamber humidity

On numerous occasions during exposure, the relative humidity in the exposure system fell below the limit set by the protocol. The actual lower limit was 26%.

On one occasion during exposure, the relative humidity was above the limit set by the protocol. The actual higher limit was 65%.

Section 8 Analysis of samples

The analytical procedure presented in Appendix 7 of the report is dated 20 October 1994, which is subsequent to the date of the analysis of the samples. This procedure was originally issued on 8 September 1994, but was re-issued at the request of the Sponsor in order to correct a number of slight changes. The term "Phosmet" replaced "IMIDAN" in the report text, and in the test article header, "IMIDAN" was replaced by "Imidan 70-WP".